Interactions between riverbed morphology, water chemistry and microbial diversity; and its impact on pollutant biodegradation

by

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Glossary of Terms

A_s	Surface area of sediment Bed
C^*	Normalised concentration
D_b	Biodiffusivity
D_d	Dispersion coefficient
D_e	Effective diffusion
D_m	Molecular diffusion
D'_m	Sediment molecular diffusion
M’	Effective Penetration Depth
t	Time
V_w	Water volume in flume experiment
β	Sediment diffusion correction term
θ	Porosity

Acknowledgements

The most rewarding part of this thesis is the ability to reminisce and look back over the course of my studies and acknowledge the support, guidance, comical genius and culinary delicacies that have been imparted upon me throughout my academic endeavours. As such, this acknowledgment section provides initial opportunity to thank those who have been most influential in encouraging me through my research.

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Declaration

I declare that the work presented in this thesis was conducted by me under the direct supervision of Professor Gary Bending and co-supervision of Doctor Jonathon Pearson, Doctor Hendrik Schäfer and Doctor Oliver Price, with the exception of those instances where the contribution of others has been specifically acknowledged. None of the work presented has been previously submitted for any other degree.

Andrew King

Abstract

Riverine environments are stressed and damaged by anthropogenic actions, with chemical contamination a key factor, identified as potentially adversely impacting the aquatic network. Standardised Organisation for Economic Co-Operation and Development (OECD) tests are used to establish biodegradation rates of chemicals in the environment, however these tests are conducted in unrealistic conditions deficient of environmental realism. Increasing the environmental realism of OECD tests has the capacity improve prediction of chemical persistence and identify potential for damage to the environment. Moreover, the interactions between bed-form, microbial biofilm communities and chemical biodegradation at the sediment-water interface are not considered in OECD tests. The current project explored the effects of bed-form characteristics on biofilm development and the consequences of sediment on biodegradation at the sediment-water interface. Novel flume systems were designed, constructed and used to develop methodologies to investigate the impact of bed form on hyporheic exchange. River water and sediment were sampled from a stretch of the River Dene (Wellesbourne, UK) and used as microbial inocula in chemical biodegradation studies using a series of specially designed re-circulating flume systems and *ex-situ* bottle experiments. Flume experimentation quantified the rate of microbial community development, topographical location and effective depth penetration on sediment beds within artificial watercourses, whilst simultaneously identifying the impact that this biofilm development possessed on hyporheic exchange. The effects of light and inoculum source on *para-nitrophenol* (PNP) biodegradation were also determined following OECD regulatory test protocols in an attempt to evaluate the realism of chemical biodegradation tests.

Experimental data showed that microbial development varied on different sediment bed profiles, and that biofilms significantly reduced the rate of hyporheic exchange on small (0.5 mm), but not large (2.0 mm) sediment particle size bed materials. Additionally, this study found that light inhibited PNP degradation in all tests and that sediment sieving utilised in OECD tests decreased rates of biodegradation of PNP. This project revealed the importance of microbial biofilms in determining hyporheic exchange. However, further experimental work is recommended to

investigate hyporheic exchange in heterogeneous sediments, whilst developing more inclusive approaches for chemical risk management by Regulatory bodies.

Chapter 1

1.1 Introduction

Anthropogenic activity can have detrimental effects upon the environment due to the intertwined nature of ecosystems (Carlow and Petts, 1992; Vitousek *et al.*, 1997). Humanity's expansion has degraded lotic ecosystems, with increased levels of industrialisation, urbanisation and land use change altering aquatic environments through elevated levels of organic and inorganic waste (Vörösmarty *et al.,* 2010); a situation that is graver in numerous regions of the developing world due to lack of suitable wastewater treatment (Finnegan *et al.*, 2009). One of the main groups of chemicals that are impacting the aquatic environment are daily use consumer chemicals that are disposed of with household waste water (Whelan *et al.*, 2011). Such chemical pollutant influx into aquatic systems can detrimentally impacts the habitability of the environment, causing shifts in biodiversity, structure and functionality of ecosystems (Lake, 2000; Dyer *et al.*, 2003).

Presently, standardised Organisation for Economic Co-Operation and Development (OECD) tests are used as regulatory tests to establish degradability of chemicals and compounds in the environment (Guhl and Steber, 2006). These tests are conducted in unrealistic conditions deficient of environmental realism, often in absence of light and using high concentrations of the test chemical (Ericson, 2007). Increasing the environmental realism of OECD tests will improve prediction of chemical persistence and help to inform potential risk to the environment (Thomas and Hand, 2011).

Research and literary evidence has highlighted the importance of multiple variable impacts in river systems such as persistence of chemicals, channel morphology and disturbance (Lake, 2000; Vörösmarty *et al.*, 2010). The extent to which pollutants carried in water come into contact with degrader communities within the sediment boundary depends on a range of factors,

including the solubility of the chemical and mixing characteristics (Dutton, 2004). The mixing characteristics may be governed by flow rate, sediment characteristics and channel morphology, particularly bed-form (Marion *et al.*, 2002; Bottacin-Busolin *et al.*, 2009). Currently, interactions between bed-form, microbial biofilm communities and chemical biodegradation at the sediment-water interface are not considered in OECD tests (Ericson, 2007). This study focuses on the effect of fluvial bed-form characteristics and microbial biofilm composition on the fate of chemical pollutant distribution and biodegradation and how they compare with OECD testing. This study assesses factors within river systems that influence soluble pollutant biodegradation, focusing on hyporheic exchange and mixing characteristics within the channel.

Hyporheic exchange occurs when the overlying water column transfers solutes into the interstitial solution surrounding porous sediments due to a concentration gradient (Cardenas *et al.*, 2004). Hyporheic exchange affects numerous environmental features, such as the flux of water exchange and provision of attenuation zones for specific pollutants by biodegradation, mixing and sorption (Packman and Brooks, 2001; Environment Agency, 2009). Consideration of hyporheic exchange is crucial when estimating contaminant fluxes in lotic systems; particularly as the bed material and bed form have the potential to control pollutant distribution patterns (Rutherford, 1994; Wondzell, 2006).

It has been suggested that microbial biofilm development possesses the capacity to impede hyporheic exchange and accelerate biodegradation rates (White, 1995). Biofilms are aggregates of microorganisms that adhere to each other on a surface within a matrix of extracellular polymeric substance (EPS), which stick to sediments within river systems. This development can affect the hydrodynamic processes in such porous media via clogging the interstitial porous spaces (Pintelon *et al.*, 2009). Clogging of this manner has the capacity to stabilize the sediment and reduce fluvial erosion of the sediment bed; additionally it is believed that the presence of biofilm may impact hyporheic exchange rates (Bottacin-Busolin et al., 2009).

Models have been created to assess risk and predict exposure distance and duration in the event of chemical spills in river systems (Technical Guidance Document, 2003 and the Impact Zone Model; see Paul *et al.,* 2005). These models are based on protocols that do not account for real world environmental variables such as biofilms and river morphology, and predominately focus on single variables.

This study investigated the potential importance of multiple variables which could affect pollutant fate in riverine systems, focusing on fluvial bed-form characteristics and microbial biofilm composition (Marion *et al.*, 2002; Bottacin-Busolin *et al.*, 2009).

1.2 "Down-The-Drain" chemicals

The chemical components used by the domestic consumer, such as surfactants, solvents, dyes, perfumes, pharmaceuticals and Triclosan (antibacterial chemical used in toothpaste and soaps), are commonly referred to as Down-The-Drain (DTD) chemicals because they are largely discharged into the sewer water system after use (Keller, 2005). Recently, there has been an increase in the awareness regarding DTD chemicals attributable to heightened concern of potential adverse human and ecological effects resulting from the employment and disposal of chemical products (Lapertot and Pulgarin, 2006). The heightened awareness around DTD chemicals is attributable to the fact that they are often disposed of with household wastewater, with no measurement or regulation of chemical quantities (Whelan et al., 2011). The biggest proportion of DTD chemicals used is within home and personal care products (HPC), such as those found in skin care products, soaps, deodorants, hair care products, household products and laundry products; quantities of which number in the thousands (Richardson et al., 2005).

Research has indicated that the global use of HPCs from 2006–2011 varied regionally with noticeable difference between developed and developing countries (url:http://www.portal.euromonitor.com/Portal/Pages/Statistics.aspx).

Recent studies have identified a geographical variance between regional HPC use, showing countrywide disparities. This is seen in the highest estimated use of HPC products in two regions in Europe, Southern England and a transboundary area in Europe containing Belgium, the Netherlands, and Germany (Price *et al.,* 2010) and variance across China, with higher usage in East and South China when compared to the Northwest (Hodges *et al.*, 2011).

The International Association for Soaps, Detergents and Maintenance Products identified that the European total household value of HPCs was estimated at 27.7 billion Euros, showing annual growth of approximately 1%. The majority of HPCs that enter the environment are laundry products, accounting for 48% of this value (url:http://www.aise.eu/).

1.2.1 Pathways of Down the Drain Chemicals into the environment

The amount of DTD chemicals reaching the lotic environment after release is predominately a function of the chemicals' partitioning behaviour and biodegradation rate. With this said, different levels of development and financial affluence affect the way in which chemicals enter the environment, meaning that there is a large geographical difference seen in water treatment. In developed countries, such as America and parts of Europe, wastewater is treated through wastewater treatment systems (Sperling *et al.,* 2002; Reemtsma *et al.,* 2008). Within typical Waste Water Treatment Plants (WWTP) processes, a measure of the solids is removed during primary treatment (40-60%), with the resulting effluent treated to remove any remaining solids prior to its subsequent release to surface water. Conversely, in the developing world, with a lack of treatment processes, DTD chemicals are released directly, straight from the household into the water system.

Primary and ultimate biodegradation occurs during transit in the sewer system, and following discharge into surface waters. Primary biodegradation is the transformation of a chemical substance by microorganisms resulting in the loss of chemical identity. Conversely, ultimate biodegradation is the breakdown of

chemical substances by microorganisms to CO_2 and readily utilisable biomolecules, and results in the production of new biomass and organic microbial biosynthesis. These HPCs are used on the human body, meaning that prior to entry to the water system they are not subject to metabolic alteration. This means that these chemicals have the potential to enter wastewater, and eventually aquatic environments, unaltered after disposal from households.

With reference to pharmaceutical use, intact and metabolized active chemicals are discharged to the aquatic environment following excretion from the body (Richardson *et al.*, 2005). The major source of human pharmaceuticals in aquatic systems originates from domestic and hospital sewage disposal, which is attributable to use of drugs for treatment of human diseases. In some cases, effluents from aquaculture systems often increase the levels of chemicals reaching the aquatic system due to the use of veterinary drugs (Boonstra *et al*., 2011).

1.3 Consequences of chemical release into the environment

Experimental research has suggested that pharmaceuticals and HPCs possess the potential to cause harm to the environment. This is often seen in the promotion of metabolic growth, development and sex alteration on the fauna and flora of environmental systems. In addition to this, DTD chemicals may result in the induction of antibiotic resistance in aquatic microorganisms and disruption of biodegradation in WWTPs (Brausch and Rand, 2011). One example of chemicals altering the environment is seen in estrogen, 17α-ethinylestradiol, which has been identified as a trace organic chemical detected in WWTP effluents in developed countries and has been linked to sex change in some fish (Nash *et al.*, 2003; Xu *et al*., 2012). Resultantly, chemical ecotoxicological data is used to establish predicted non-effect concentrations (PNEC), whilst chemical behaviours are assessed to determine the predicted environmental concentration (PEC). These two variables are compared to identify whether the influx of the test substance is a potential environmental risk by establishing whether the PEC is higher than the PNEC (Pagga, 1997).

Chemical environmental fate pertaining to Pharmaceutical Personal Care Product (PPCP) chemicals is starting to become more widely appreciated and understood, however it remains to be adequately documented. However, some persistence data and bioaccumulation values in aquatic organisms have started to be reported in more depth. The widespread employment of antibiotics, for example, is a major cause of the spread of resistance among bacterial pathogens. Exposure to antibiotics can alter the structure of microbial communities and thereby affect the higher food chain (Daughton and Ternes, 1999). Government and industrial sponsors have designed legislation to manage and control the generation, distribution and vending of domestic and personal care products that use persistent, bioaccumulative or toxic chemicals, all of which has resulted in heightened levels of monitoring and chemical investigation.

1.4 Regulatory drivers for chemical management

Chemical regulation is required to monitor the influx and abundance of substances in the environment, in addition to reviewing their application, quantities and concentrations measured in the environment. Anthropogenic activities, particularly those linked to development, have resulted in higher levels of chemical employment, thereby calling for a greater level of understanding of the chemical dissipation processes occurring in the environment.

1.4.1. REACH and PBT assessment

Drawbacks associated with previous assessment of new and existing chemicals have resulted in the European Union creating new regulations for the registration, evaluation, authorisation and restriction of chemicals (REACH) (Ahlers *et al.*, 2008).

To achieve enhanced and more robust assessments whilst reducing animal testing, information has to be used in an integrated manner. REACH Implementation Projects updated and extended technical guidance documents for risk assessment of new and existing chemicals. This legislation has resulted in new levels of innovation, resulting in the design of safer chemicals and processes that have enhanced corporate responsibility and promoted competition within the European Chemical Industry (Rudén and Hansson, 2010).

Knowledge of the biodegradability of chemicals is an essential requirement for hazard, environmental risk and persistence assessment (ECETOC, 2007), resulting in the production and evolution of international and national frameworks to identify PBT substances and control their emission into the environment. Understanding the mechanisms responsible for chemical persistence (intrinsic, environmental and physical persistence) is therefore important for assessment.

Subsequently, REACH set minimum data requirements to obtain high levels of protection for human health and the environment, which call for adequate standardized information to draw overall conclusions with respect to the regulatory endpoints classification and labeling, for persistent, bioaccumulative and toxic (PBT) assessment and predicted no-effect concentrations (PNEC) derivation (Ahlers *et al.*, 2008).

1.5 Determination of Biodegradation Rates

Environmental risk of particular chemicals is established via estimation of their likely concentrations in the environment (OECD Guideline for testing of chemicals, 2005). Chemical biodegradability is the most important aspect of establishing chemical environmental behaviour and is essential for chemical classification purposes (Rudén and Hansson, 2010). As such, biodegradation rates are determined by employment of the chemical knowledge of the likely

use and disposal patterns, identification of the physico-chemical properties and the characteristics of the receiving environment.

1.5.1 Predictive Modelling

Prediction of chemical biodegradability in the natural environment is one of the main focuses of researchers as the ability to predict the chemical persistence in the environment lessens the necessity for experimental testing. Several computer models have been generated to predict biodegradability based on a series of calculations that identify the probabilities of individual chemical transformations. CATABOL, for instance, calculates the environmental persistence of chemicals while also generating information on metabolic pathways and identification of stable metabolites (Jaworska *et al.,* 2002). Whereas BIOWIN models estimate the probability of rapid aerobic biodegradation, a model that has been partially validated in subsequent research (Boethling *et al.*, 2003; Posthumus *et al.,* 2005).

1.5.2. OECD tests

Ranges of methods for investigating biodegradation processes have been developed to predict chemical fate in the environment. To predict environmental fate of chemicals the Organisation for Economic Cooperation and Development (OECD) established standardised laboratory methods to estimate environmental biodegradation. However, these tests are conducted in unrealistic conditions deficient of environmental pragmatism. They are undertaken in the dark with unrealistically high levels of the test compound, and with unstandardized inoculum. This means that the tests are conducted without consideration of the inoculum size, origin or diversity of the microbial community involved in biodegradation, meaning that their outputs are not capable of predicting the rate of biodegradation in the natural environment. Increasing the environmental realism of OECD tests will improve prediction of chemical persistence and identify potential for risk to the environment.

1.5.3 Overview of Current Testing

1.5.3.1 Ready Biodegradability Tests

Ready biodegradability tests are first tier tests, identified as providing only limited opportunities for biodegradation and acclimation of the inoculum (Reuschenbach *et al.*, 2003). These tests are conducted in aerobic conditions using high concentration levels of the test substance (OECD Guidelines for testing of chemicals, 1992). The test pass levels have to be reached within 10-days of the 28-day test period. These types of test permit identification of a test compound's degradability under natural conditions, with a pass indicating the test chemical is degradable. However, this type of test has been suggested to underestimate the potential of degradation in actual environmental systems (Lapertot and Pulgarin, 2006; OECD Guidelines, 2005).

1.5.3.2. Inherent biodegradation tests

The next levels of testing are inherent tests (second tier). These tests load the experimental systems with high concentration levels of the target compound and the inoculum under aerobic conditions (Comber and Holt, 2010). Inherent biodegradability is measured by either primary biodegradation (specific analysis) or by ultimate degradation (non-specific analysis), although these tests do not restrict the length of test duration or specify the biodegradation rates. Biodegradation levels that surpass 20% of the theoretical value are evidence of inherent, primary, biodegradability, although biodegradation above 70% of the theoretical is taken to be evidence of inherent, ultimate biodegradability. This higher tier test provides a higher probability of detecting biodegradation compared to first tier tests for ready biodegradability, meaning that a negative test result under these conditions may indicate potential environmental persistence (Comber and Holt, 2010).

1.5.3.3 Simulation tests

Simulation tests are conducted in aerobic and anaerobic conditions, designed to provide data for chemical biodegradation rates under specified environmentally relevant conditions. These are provisionally designed to replicate and assess the rate and extent of biodegradation in either the aerobic treatment stage of a WWTP or environmental compartments. The tests add an additional layer of testing, incorporating indigenous biomass, media, or relevant solids (e.g. soil, sediment) to assess sorption of the test chemical and replicate a particular set of environmental conditions. These tests are designed to employ biodegradation kinetics that mimic the actual environmental conditions more closely, employing lower concentrations of the test chemical (e.g. less than 1 µg/L to 100 µg/L).

Chemical fate in WWTPs is often investigated in the laboratory setting via employment of simulation tests, particularly those that assess the impact of Activated Sludge Units (OECD 303 A) and Biofilms (OECD 303 B) on experimental chemicals. Monitoring changes in Dissolved Organic Carbon (DOC) and/or Chemical Oxygen Demand (COD) identifies the biodegradation of the test chemical (Comber and Holt, 2010).

1.6. Environmental realism of tests

There are a plethora of OECD tests designed to replicate different environmental conditions, such as those employed to reflect terrestrial compartments (OECD 307) and aquatic compartments (OECD 309, OECD 314). These tests are based on static, semi-continuous or continuous principles, operated under aerobic or anaerobic conditions with different test media. During experimentation the test conditions are standardized as much as possible and are set to reflect the environmental compartment and the biodegradation processes that occur within it.

It is possible to conduct higher tier biodegradation tests to identify the biodegradation kinetics of test substances; the duration of these tests together with their cost and complexity reduces their suitability as standardised protocols (Merrettig-Bruns and Jelen, 2009). Resultantly, OECD tests are conducted to assess the biodegradation rates of test chemicals, however they do not take into account the variations seen in natural environments, particularly the water chemistry, inoculum concentration and composition, environmental context, the test substance concentration or the global relevance.

1.6.1. Effect of environmental factors on biodegradability assessment

It has been noted that chemical degradation screening tests categorise readily biodegradable compounds from persistent and there is suggestion that they often underestimate the potential of chemical degradation in environmental systems (Guhl and Steber, 2006). This underestimation could be attributable to a plethora of factors, ranging from the differences between *in-situ* and *ex-situ* experimentation due to variations between lab and natural environments, such as presence/absence of light, oxygen, pH, flow rate and river bed form. There are countless experimental studies that have illustrated examples of factors that occur in natural environments and the way in which they potentially affect the biodegradation of chemicals in laboratory conditions. Oxygen, for example, has been identified as a key driving force behind chemical biodegradation. Studies have shown that the presence or absence of oxygen has different effects on chemical degradation, seen in the anaerobic degradation of phenol in digested sludge found by Boyd *et al.* (1983), compared to the aerobic degradation of *para*-nitrophenol (500 mg/L) by activated sludge (Bhatti *et al.*, 2002). In addition to this, bed-form has also been found to alter the biodegradation of chemicals, with the effect of flow velocity and sediment dynamics, particularly the water-sediment interactions and bed-form morphology, being found to impact the attenuation of acidic pharmaceuticals, the majority of which degraded within 2.5 - 18.6 days (Kunkel and Radke, 2008). Moreover, other environmental factors have been found to impact biodegradation, shown in the work of Li *et al.,* 2008, who found that degradation of para-nitrophenol would occur by an *Arthrobacter*

sp. isolate within 120 hours if not impacted by pH (optimum pH 8-9). It has also been discovered that longer degradation times occur at lower temperatures (Kang and Kondo, 2002).

The rationale behind generating a standardised test protocol for chemical degradation studies that omit variability in environmental parameters is to simplify the test system so that it is not impeded by variables that will not be replicated in all environmental systems, thereby standardising its application possibilities. For example, light is not present in biodegradation tests to avoid algal development and prevent it from affecting the biodegradation of tested chemicals or alter the possible interpretation of CO_2 data analysed in such tests due to fixation. There are an abundance of environmental parameters that vary from watercourse to watercourse, making standardisation of testing necessary. However, in order to standardise testing to permit 'accurate' modelling of chemical biodegradation, a plethora of environmental parameters are not taken into consideration. These parameters include temperature, which affects bacterial growth and biomass (White *et al.,* 1991), pH which may alter the toxicity of environmental pollutants (Rutgers *et al.*, 1998; Li *et al.*, 2008), chemical mineralization (Boyd *et al.,* 1983), channel morphology and channel flow dynamics that impact contact of chemicals with bacteria on the sediment surface (Kunkel and Radke, 2008) and the biofilm community structure and function (Battin *et al.,* 2003).

1.6.2. Water chemistry

Across the length of an impacted river the biota 'self-purifies' the environmental compartment, degrading chemicals that enter the water column. This biotic recovery is the river's natural resilience to environmental disturbance, often dependent on increases in Dissolved Oxygen (DO) and nitrification. The further downstream from the point source origin of the contaminant within the water body, the greater the recovery (Dyer *et al.*, 2003), illustrated in Figure 1.1. Quantification of this capacity for self-purification can be used to gauge the

potential effects of DTD chemicals that enter watercourses (McAvoy *et al.*, 2003).

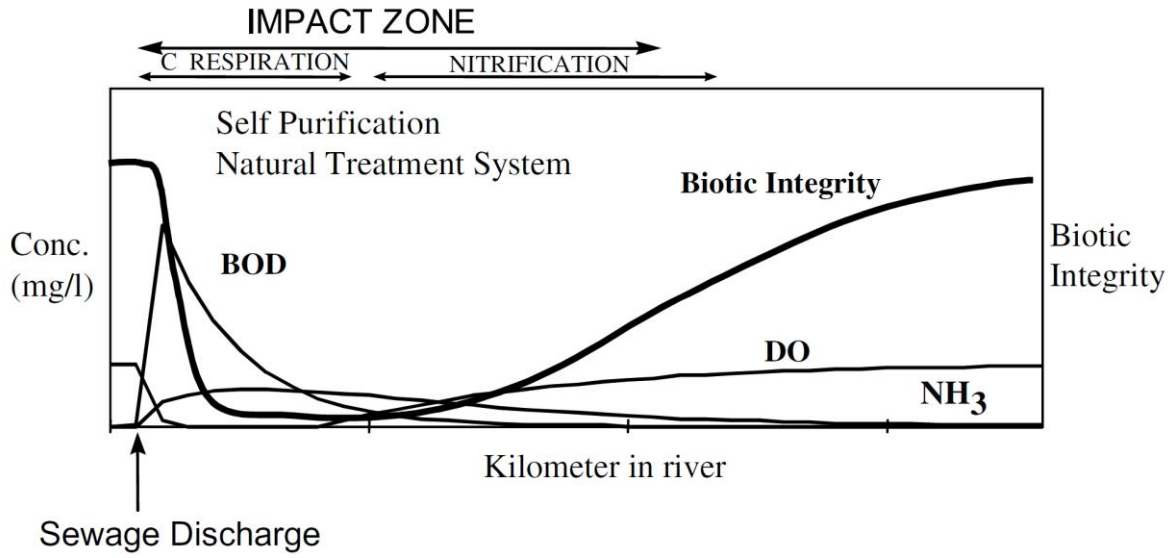


Figure 1.1: Relationship of impact zone caused by direct discharge of organic wastes (Source: Dyer *et al*., 2003)

1.6.3. Inoculum

Ex-situ laboratory studies employed to assess chemical biodegradation rates in the environment are most realistic when conducted using mixed culture systems extracted from the field (Spain and Veld, 1983). However, numerous experimental factors must be controlled when running biodegradation studies as the biodegradation rate can be impacted by variance in experimental factors, such as temperature, pH, salinity and microbial biomass (Spain and Veld, 1983; Thouand *et al.*, 1995). If laboratory studies are to be used to predict biodegradation rates in the field, it is important that the rate-determining factors are understood (Spain and Veld, 1983) and controlled (Thouand *et al.*, 1995).

Previous studies have identified the necessity to control the physio-chemical conditions (pH, temperature, concentration of mineral elements) and the concentration of the test substance (Thouand *et al.*, 1995). It has also been identified that the bacterial inoculum concentration should be controlled to ensure a reproducible and representative test (Thouand *et al.*, 1995). It has been possible to control the physiological and chemical concentration of biodegradation tests due to the simplicity of the experimental design. However, maintaining and establishing a reproducible inoculum concentration is extremely difficult as the characteristics of the inocula (presence or absence of biodegraders, cell density, presence of protozoa), which control the biodegradation kinetics and the lag phase, can lead to problematic interpretation due to high variability in the biodegradability determination of chemicals (Thouand *et al.*, 1995). As such, tested inocula should be characterized by their size and biodegradation potential. Regrettably, approximation of bacterial density is rarely undertaken as there are presently no accessible, practical methods to measure inoculum diversity in industrial laboratories, meaning that advancements are needed to ensure accurate testing (van Ginkel *et al.,* 1995).

Additionally, previous work has shown that adaptation of microorganisms can also play a major role in determining biodegradation rates (Feslet *et al.,* 1981). Adaptation is defined here as a change in the microbial community that increases the rate of transformation of a test compound as a result of a prior exposure to the test compound. All of these variables result in differences in inocula used in simulation tests. As biodegradability tests may involve inoculum from different origins, inocula source should also be taken into account while running tests (Thouand *et al.*, 2011) as surface water, sediment and soil samples all support different community structures of microbial composition, diversity and abundance. The influence of this inoculum variability on biodegradation tests is attributable to the source and concentration of live bacteria, the adaptation of bacteria to the test molecule and the presence of protozoans from the natural environment (Barkay and Pritchard, 1988; Thouand *et al.*, 1995). It is therefore imperative to control the number of specific biodegraders used in biodegradation tests to limit the risk of errors in biodegradability tests (Thouand *et al.*, 1995; Forney *et al.*,2001).

1.6.3.1. Biofilm as inoculum

The microbiology of rivers and streams, and its subsequent impact on biodegradation, has been very much neglected (Neu and Lawrence, 2006). This is most likely attributable to the difficulty associated with *in-situ* study and/or the use of laboratory models of lotic systems (Left, 1994). Irrespective of this, the interfaces in these constantly changing ecosystems contribute significantly to the geographical distribution and constant recycling of contaminants and nutrients in all flowing water systems (Lock, 1993; Neu and Lawrence, 2006).

Stream ecosystems are complex and diverse in nature due to the constant and endless influxes of nutrients, chemicals and energy flows that enter the water-sediment system at any point along the channel length from a variety of sources. As a result, stream biofilms are generally complex communities composed of bacteria and algae embedded in an extracellular polysaccharide matrix that is often attached to substrates such as sediments and aquatic plant

surfaces (Vinten *et al.,* 2011). Research conducted on the presence of biofilm communities within lotic systems has identified these communities as an influencing factor in the removal of contaminants from the water column, thereby enhancing the self-purification of surface waters (Edwards *et al.*, 1990).

The development of biofilm communities on the sediment bed results in them acting as the first boundary that interacts with dissolved substances entering and being transported along the water column. Biofilms possess the capacity to integrate the effects of environmental conditions over extended periods of time due to their rapid growth rates and the physiological variety of the organisms from which they are formed. As such, standardized methods of biofilm extraction from river ecosystems could be applied to biodegradation testing where biofilm is employed as inoculum, as numerous studies have identified biofilms as possessing a significant impact on the rate of chemical biodegradation (Araya *et al.*, 2003; Tien *et al.*, 2011). Moreover, as chemical biodegradation is often species specific, requiring a certain species of bacteria to degrade a specific chemical, biofilms possess the capacity to act as natural microbial populations in testing due to their reflection of the actual river environment (Thouand *et al.*, 2011). Furthermore, modifications in bacterial communities may be attributable to the disruption of important ecosystem processes which have the capacity to impact chemical presence in water/sediment systems. As such, detection of microbial community modifications is important for monitoring the effects of chemicals in river ecosystems, and in turn, enhancing chemical risk assessment.

1.7 Hyporheic Exchange

The hyporheic zone is the band of saturated, permeable sediment surrounding a river's water column, where surface water and groundwater mix (Tonina and Buffington, 2007). This zone encompasses all aspects of the surrounding river sections, ranging from the riverbeds and banks to the saturated sediments and floodplain areas (Edwards, 1998). This is one of the most physically active and

diverse zones within lotic systems, where active mixing occurs that follows chemical gradients due to the mixing of groundwater and surface water by fluvial fluxes, all of which supports an ecotone of benthic macroinvertebrates and fish (Gibert et al., 1994; Williams and Hynes, 1974). The speed and extent of hyporheic exchange is governed by flow interactions with the channel morphology and the resultant rate of hyporheic exchange (Harvey and Bencala, 1993; Elliott and Brooks, 1997).

Stream-subsurface exchange processes are important because of their role in controlling the transport of contaminants and ecologically relevant substances in streams (Packman *et al.*, 2004). These exchanges have the capacity to affect numerous environmental features, ranging from controlling the flux of water exchange to the provision of attenuation zones for specific pollutants by biodegradation, mixing and sorption (Packman and Brooks, 2001; Environment Agency, 2009).

Stream-subsurface exchange is receiving increased investigation due to intensified awareness of its control of contaminant and nutrient material fluxes (Marion *et al.*, 2002). This sediment-water interface is an assemblage of biological communities, mineral particles, organic material and void spaces forming a boundary for fluxes of solutes, water and particulates (O'Connor and Harvey, 2008). The bed acts as a transient storage mechanism for solutes, increasing retention time within the system (Bencala and Walters, 1983). The rate of channel-bed penetration exchanges is of growing interest and its analytical methodology has been reviewed in depth (Battin and Sengschmitt, 1999; Bottacin-Busolin *et al.,* 2009; O'Connor and Harvey, 2008; Marion *et al.,* 2002).

1.7.1 Solute Transport

Solute transport is one of the most crucial driving forces in riverine systems, influenced by chemical, biological and physical processes, the magnitude of which is dependent upon both the environmental parameters and the solute properties (Ng and Yip, 2001). Following point source pollution events, modelling identifies the point downstream that pollutant will cease to affect the watercourse (Dutton, 2004). This is affected by solute transport mechanisms which impact the rate of hyporheic exchange of soluble particulates from the overlying water column to the interstitial fluid in the sediment bed across a sediment-water interface (O'Connor and Harvey, 2008). There are two processes that operate to mix suspended solutes: advection and diffusion. Advection is the bodily movement of a mass of fluid by flow regime, changing physical or chemical properties of the fluid (Chanson, 2004). Diffusion is the movement of liquid particles as a result of thermal agitation from an area of high concentration to an area of lower concentration (Chanson, 2004); this can be either molecular or turbulent. Advective transport moves the solute away from the source point with bulk fluid movement and thus providing the suspended particulates similar velocities to the fluids in the channel (Ng and Yip, 2001). Diffusion spreads the solute transversely and longitudinally throughout the channel along a concentration gradient resulting from turbulence, molecular and/or effective diffusion (Chanson, 2004).

1.7.2 Diffusion

Following the infiltration of a neutrally buoyant solute into an open channel it is acted upon by two processes; advection and diffusion (Dutton, 2004). Advection carries the solute away from the source whilst diffusion whilst diffusion spreads the solute across and along the channel. There are two types of diffusion, molecular and effective.

1.7.2.1 Molecular Diffusion

Molecular diffusion occurs in stationary fluids when high concentrations of solute are added to the overlying water column, creating a concentration gradient causing the solute to spread in all dimensions due to random molecular movement, eventually extending across the sediment-water interface and spreading through the pore water (Rutherford, 1994). Rutherford (1994) states that Fick's first law explains this diffusion as the rate of particle movement is comparative to the spatial concentration gradient (O'Connor and Harvey, 2008).

The rate of molecular diffusion through the hyporheic layer is directly affected by sediment, as solute must navigate the sediment particles to gain access to the pore water, reducing the molecular diffusion coefficient. Theoretical and empirical studies show molecular diffusion coefficients (D'_m) are calculated by taking porosity and tortuosity into account (Berner, 1980), thereby generalising the sediment molecular diffusion expression to:

$$D'_m = \beta D_m$$

Where D_m is the rate of molecular diffusion and β (sediment diffusion correction term) signifies the empirical expression for tortuosity as a function of porosity (θ) (see Iversen and Jørgensen, 1993, for further description).

1.7.2.2 Effective Diffusion

Turbulent diffusion is very efficient in rapidly decreasing the concentrations of contaminants that are released into the natural environment (Roberts and Webster, 2002). Effective diffusion amalgamates physical transport mechanisms within the fluvial system to generate a coefficient for overall solute exchange (O'Connor and Harvey, 2008). This exchange coefficient includes effects of bioturbation, turbulent diffusion, dispersion and molecular diffusion as

natural systems are complex and a combination of processes cause net hyporheic exchange. These processes include molecular diffusion, advective pumping, sheardriven flow, turbulent surface penetration and hyporheic water mixing (O'Connor and Harvey, 2008). Presently, no singular theory exists to predict the resulting exchange in terms of bed parameters or measured streams. Instead, bulk solute exchange across the sediment water interface can be modelled with an effective diffusion coefficient that includes all the factors that influence hyporheic exchange. This can be shown formulaically by:

$$D = \beta(D_m + D_b) + D_d$$

Where: D_b is the biodiffusivity and D_d is the dispersion coefficient, encompassing turbulent diffusion and pumping (Berg et al., 1998).

According to O'Connor and Harvey (2008), it is possible to obtain a measured value of effective diffusion using a tracer, where hyporheic exchange forces the tracer placed in the channel into the pore spaces of the sediment bed, resulting in fluid exchange from the bed sediment to the water column over time.

O'Connor and Harvey's (2008) analysis of hyporheic exchange investigations identifies a scalar relationship to predict the rate of mass transfer in near surface sediments which is applicable to all flow and substrate parameters. This data quantifies net hyporheic exchange using an effective diffusion coefficient incorporating all the transport processes, using dimensional analysis to scale effective diffusion to shear stress velocity, roughness height and permeability to describe sediment and flow characteristics (O'Connor and Harvey, 2008). This analysis created the effective diffusion (D_e) formula for tracer experimentation, providing a representative analytical calculation for hyporheic exchange:

$$D_e = \left(\frac{\sqrt{\pi}}{2}\frac{V_w}{A_s}\frac{dC^*}{d(t^{1/2})}\right)2$$

Where V_w is the volume of water, A_s is the surface area of the sediment bed, C* is the normalised concentration and t is time.

1.7.3 Pumping

Changes in channel bed-form cause fluctuations in pressure distribution over the bed. Changes in bed topography modify the flow gradient and create pressure variations which cause pumping in porous bed sediments, a process which alters flow through streambed sediment (Packman and Bencala, 2000). Channel flow impediments, such as bed-forms, create high-pressure regions upstream of the obstruction and low pressure downstream. This pressure gradient results in the overlying water being driven into the interstitial porous spaces within the sediment bed store, travelling through the obstruction and then exiting downstream of the obstacle. These processes, known as upwelling and downwelling correspondingly, create hyporheic circulation under the obstruction (Tonina and Buffington, 2007). This process is illustrated in Figure 1.2, showing tracer movement though bed-form, driven by pumping flow. The presence of bed forms such as dunes, therefore, theoretically alters the rate of mixing within the channel, affecting mass transfer into the bed (O'Connor and Harvey, 2008).

Models that predict mass transfer into the sediment (Elliot and Brookes, 1997) by pumping have been devised in order to quantify hyporheic exchange by examining the sediment domain. O'Connor and Harvey (2008) built on this and created a formula to calculate the effective depth of solute penetration as a result of pumping, using the same parameters used to derive effective diffusion which considers amplitude, wavelength and particle size.

$$D_e = \left(\frac{\sqrt{\pi}}{2}\frac{d(M'/\theta)}{d(t^{1/2})}\right)^2$$

Where M' is the effective penetration depth and θ is porosity.

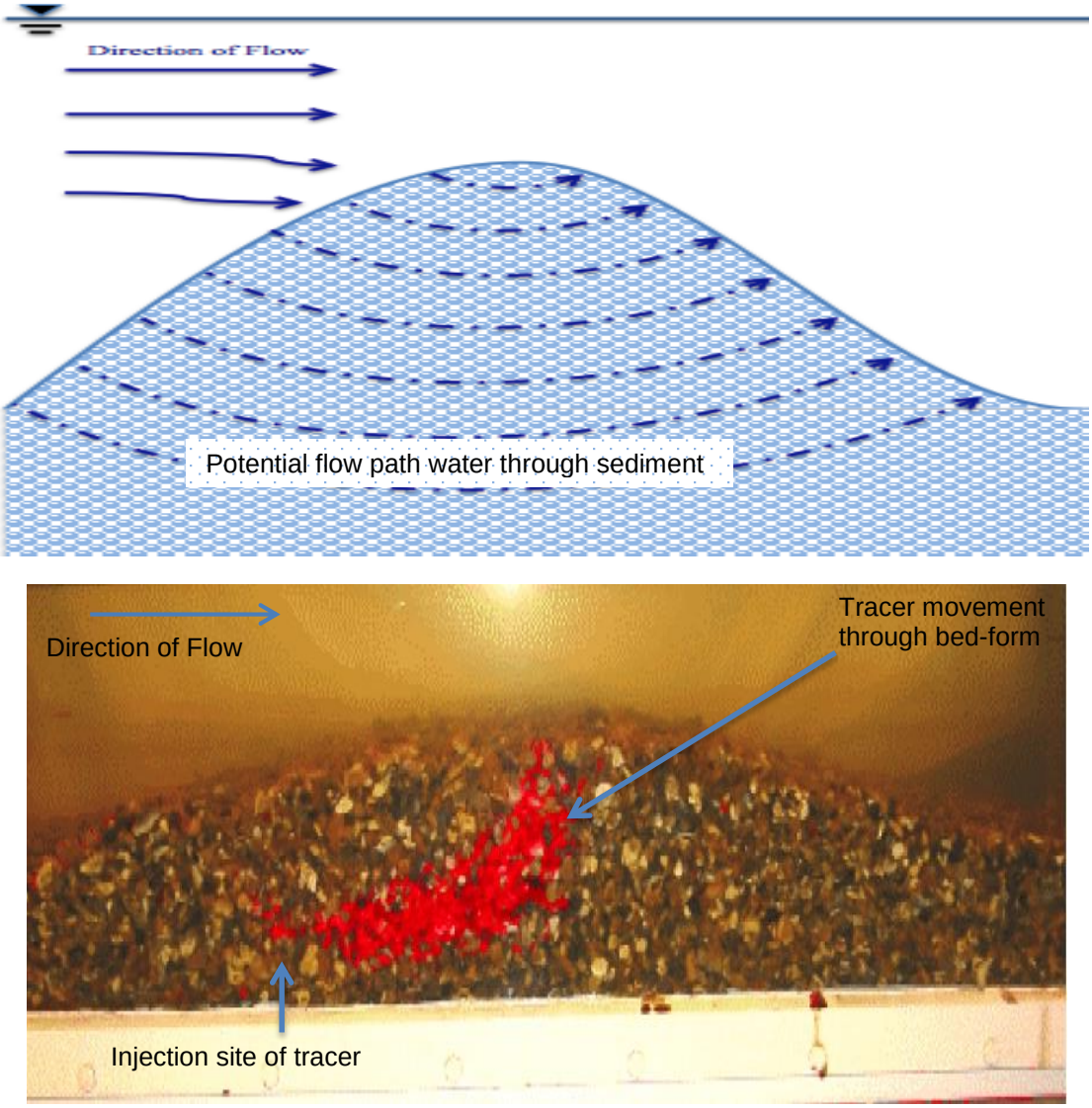


Figure 1.2: Schematic example of potential pumping movement through a bed-form compared against an enhanced experimental tracer photograph of a similar bed-form, illustrating the tracer movement through the bed via pumping (image taken from Dutton, 2004).

1.7.4 Previous Experimental Testing analysing exchange

The following sections identify previous experimental study into hyporheic exchange, both in the laboratory and in the field. The majority of the laboratory studies detailed were incorporated into O'Connor and Harvey's [2008] effective diffusion scaling relationship.

1.7.4.1 *In-situ* Testing

There are a limited number of *in-situ* field experiments that have tried to assess hyporheic exchange when compared to laboratory investigations. The favoring of *ex*-situ testing is due to the complexity surrounding the environmental system, particularly in terms of quantifying all of the parameters that influence the exchange and generating a method to accurately measure exchange with all the other variables present. The majority of previous investigations used transient storage models to determine the hydraulic parameters and mixing coefficients, including hyporheic exchange.

Tracer experiments have been conducted in numerous rivers across the globe in an attempt to quantify hyporheic exchange. Examples of such studies are the tests conducted in Uvas Creek in Sierra Nevada, California, by Bencala and Walters [1983], the Sava Stream in Sweden (Johansson et al., 2001; Worman et al., 2002; Forsman et al., 2002) and reaches of St. Kevin Gulch in Colorado (Kimball et al., 1994). All of these studies have attempted to quantify the factors of influence that drive and impact hyporheic exchange, however the complexities and unpredictability of the environmental conditions in the lotic systems resulted in these tests only providing a glimpse into hyporheic exchange.

1.7.4.2 *Ex-situ* Testing

Due to the environmental variability of *in-situ* testing, *ex-situ* experimentation has been favoured due the investigators' ability to more closely control and monitor the experimental parameters. This can be seen in the employment of flume systems in the vast majority of modern experimental work, in which all parameters are maintained to permit the isolation and investigation of one variable.

This can be seen in the work of Dutton (2004), who maintained all experimental parameters as constant during testing apart from the bed-form; permitting analysis of bed-form in large scale flume tests. Conversely, the work of Chandler (2012) assessed the impact of sediment size and velocity on exchange; employing a novel system that permitted alteration of a single variable at a time, thereby removing experimental variance that would otherwise be found in natural systems.

1.7.5 Predicting Scaling Relationships

A plethora of methods have been proposed for accurate modelling of hyporheic exchange (see Packman and Bencala, 2000). However, these methods do not take flow variations or alterations in sediment characteristics into account, resulting in disparities between models and recorded data. Resultantly, O'Connor and Harvey (2008) collated data from hyporheic exchange flume experiments to create a data set encompassing a large spread of flow and sediment conditions. A measured effective diffusion coefficient was calculated for each study, however the varying experimental systems required different calculations to be employed based on injection and sampling locations (i.e. bed or channel).

O'Connor and Harvey (2008) specified scaling constant and exponents by plotting three dimensionless numbers against the dimensionless diffusion

coefficient, which demonstrates a strong correlation between the Péclet permeability number and shear Reynolds number.

1.8 Biofilms

The formation of biofilms is a complex multi-functional process in which microorganisms grow on a surface and produce extracellular polymers, resulting in alterations in the organism phenotype (Singh *et al.*, 2006). In rivers, biofilms are predominately attached to sediments. The formation of a functional biofilm (Figure 1.3) starts with irreversible attachment and establishment, followed by maturation and finally detachment (Madigan and Martinko, 2006). Biofilm formation and development is regulated by environmental factors like pH, nutrient and oxygen availability; which all cause biofilm variance (Fux, 2005).

Biofilms enhance microorganisms' chances of survival and development as embedment within the adhesive EPS matrix results in an altered phenotype with growth rate and gene transcription (Denyer *et al.,* 1993). Organisms within biofilms communicate and coordinate their actions using signalling molecules known as quorum sensors (Kjelleberg and Molin, 2002). This structural communication enables dispersal of nutrients when there is heightened competition for food at high cell densities within the biofilm (Stanley and Lazazzera, 2004). Amalgamation of bacteria into microbial communities enables them to withstand increases in shear forces, pH changes, nutrient deprivation and chemical fluxes due to mechanical strength and support provided by the EPS (Davey and O'Toole, 2000), whilst also transferring exopolysaccharides to higher trophic levels (Decho, 1990; Denyer *et al.,* 1993).

Biofilm systems adapt and survive due to their enclosed nature. The physiological properties of the microorganisms, such as biosurfactant production and chemotaxis, enhance bioavailability and degradation of hydrophobic compounds (Singh *et al.*, 2006).

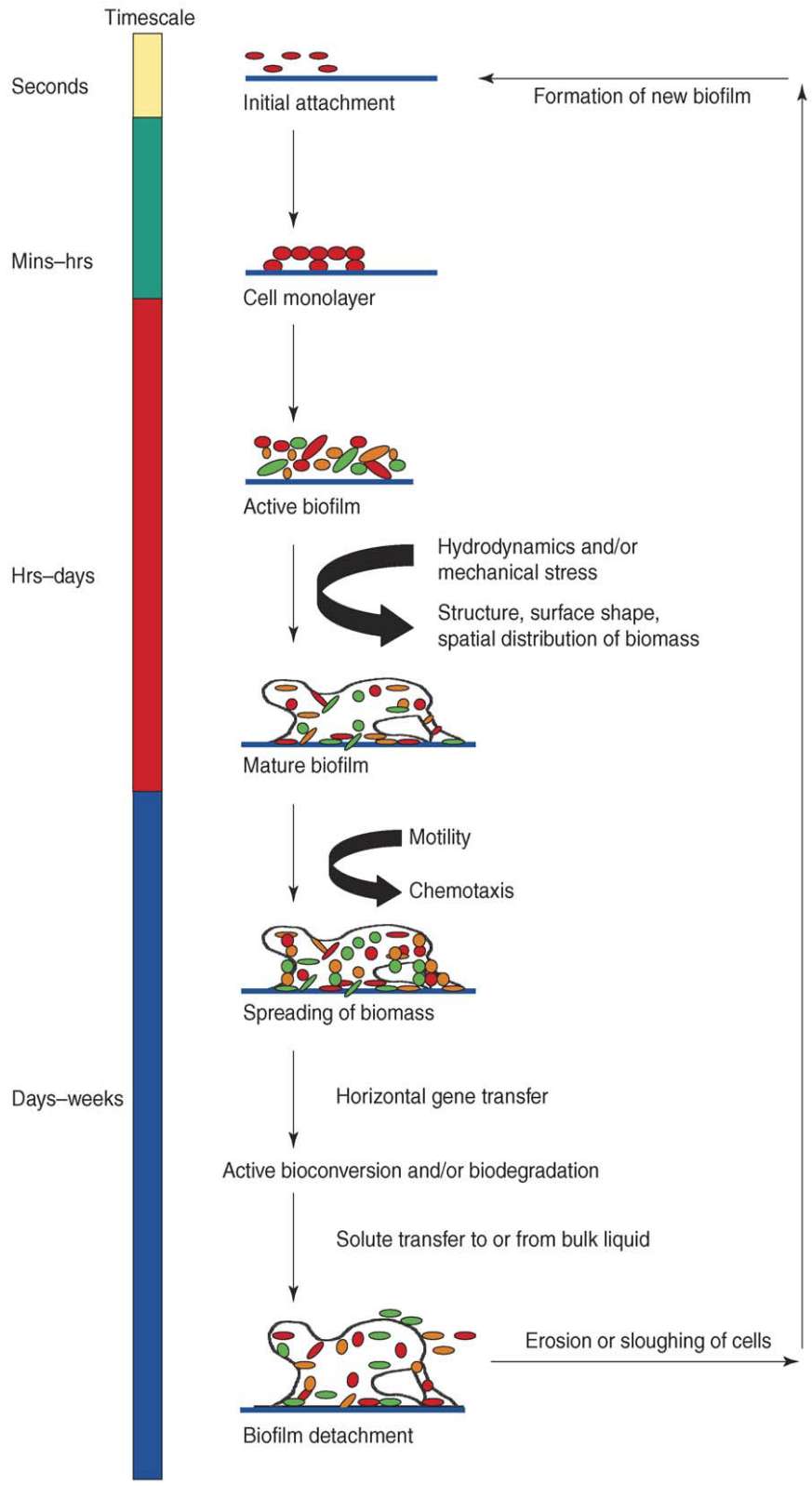


Figure 1.3: Illustration describing stages involved in biofilm formation (Taken from Singh *et al.*, 2006)

The dense, packed structure of biofilms result in autocatalytic promotion of gene transfer, enhancing the physiological and catabolic performance of complex communities by the formation of new degradation pathways (Denyer *et al.*, 1993; Fux, 2005; Singh *et al.*, 2006). This transfer of plasmids may encode beneficial traits, such as the ability to degrade pollutants, thereby making biofilms useful as a form of bioremediation (Decho, 2000; Fux, 2005).

Increased realism is required to accurately model chemical fate in the environment, particularly accounting for the impacts of hydraulic exchange, bed-form variation and biofilm development. This is because it is believed that biofilm development can affect hydrodynamic processes in porous media such as riverbed sediments, through the process of clogging interstitial porous space (Battin and Sengschmitt, 1999).

1.9 Study Objectives

Studies of lotic systems are regularly identifying rivers as biogeochemical hotspots that possess the capacity to process influxes of organic matter that enters the aquatic environment (Battin et al., 2009; Mulholland et al., 2008). Research has suggested that most biological and chemical interactions occur within the hyporheic zone, with suspended solutes interacting at the water-sediment interface. The sediments at this interface act as host for microbial biofilm colonisation, which have the potential to influence biogeochemical transformations within the river (Battin et al., 2008). This finding has identified the need to research the variables controlling microbial community development.

Provisional flume testing and other research, such as that of Nogaro et al. (2010), has indicated that microbial community abundance and distribution differs on sediments with different sized particles. The variance in microbial community development fluctuates drastically when *in-situ* heterogeneous sediments are examined, making it difficult to quantify the impact of biofilm development on exchange (Nogaro et al., 2010). Moreover, mixing characteristics are governed by flow rate, sediment characteristics and channel morphology. Currently, interactions between bed-form, microbial biofilm communities and chemical biodegradation at the sediment-water interface have not been studied in depth. To reach a stage where enhanced modelling can be conducted, experimentation and data collection must take place. This investigation aims to explore the effect of bed-form characteristics on the diversity and pollutant-degrading potential of microbial biofilm communities at the sediment-water surface, and the subsequent impact biofilm development and bed-form has on hyporheic exchanges. The aims assessed in this research project are:

1) Establish an experimental system and methodology capable of assessing solute exchange in a novel flume system and generate reproducible data (**Chapter 2)**

2) Assess the impact of bed-form on the rate of hyporheic exchange and identify the impact biofilm presence possesses on the rate of hyporheic exchange **(Chapter 3)**

3) Quantify the environmental realism of OECD chemical biodegradation tests **(Chapter 4)**

Chapter 2

Flume Development

2.1 Introduction

Hyporheic exchange occurs when the overlying water column transfers solutes into the interstitial solution surrounding porous sediments due to a concentration gradient (Cardenas *et al.*, 2004). Such stream-subsurface exchange processes are important because of their role in controlling the transport of contaminants and ecologically relevant substances in streams (Packman *et al.*, 2004). This exchange affects numerous environmental features, from controlling the flux of water exchange to provision of attenuation zones for specific pollutants by biodegradation, mixing and sorption (Packman and Brooks, 2001; Environment Agency, 2009). Such exchange is governed by flow rate, sediment characteristics and channel morphology (Rutherford, 1994). Consideration of hyporheic exchange is crucial when estimating contaminant fluxes in lotic systems; particularly as the bed material and bed form have the potential to control pollutant distribution patterns (Rutherford, 1994; Wondzell, 2006).

Currently, interactions between bed-form, microbial biofilm communities and chemical biodegradation at the sediment-water interface have not been studied in depth. To reach a stage where accurate modelling of exchange processes can be conducted, experimentation and data collection must take place.

Experimental studies examining hyporheic exchange have been conducted for over a century; however understanding fluvial processes has been restricted by the complex conditions found in natural environments (Friedrich *et al.*, 2007). Resultantly, numerous studies have resorted to employing artificial watercourses (flumes) in the laboratory to gain a better understanding of the

role of individual factors affecting exchange processes in river systems (Williams, 1970). Additionally, recent *in-situ* riverine experimental studies have emphasised the necessity to conduct more in depth experimental studies to explore the consequences of microbial sediment clogging in the hyporheic zone (Nogaro *et al.,* 2010).

Laboratory flume studies permit simulation of natural environments in a controlled manner, using water and sediment in quantities small enough to control (Friedrich *et al.*, 2007; Williams, 1970). Natural riverine environments contain different boundary conditions than those typically found in laboratory flumes, however the unsteady flow conditions, intermittent sediment and water supplies, nutrient fluxes and other uncontrollable variables in different rivers prevents controlled experimentation (Friedrich *et al.*, 2007). Conversely, laboratory based studies permit efficient investigation of water-sediment interaction under controlled conditions, which can then be used to predict fluvial responses to specifically predefined criteria.

The aims of the work presented in this chapter were:

1) Design and build a flume system suitable for investigating hyporheic exchanges
2) Develop methodologies for assessing hyporheic exchange in the flumes
3) Establish the reliability and reproducibility of the flume system

2.2 Materials and Methods

Each laboratory possesses custom built equipment to suit its particular interests, capacity, and needs (Williams, 1970). The majority of hyporheic exchange experiments conducted in the last two decades have been conducted in re-circulating flumes (e.g. Elliott and Brooks, 1997; Marion *et al.,* 2002; Richardson and Parr, 1988; Tonina and Buffington, 2007). Fluvial experiments reported in the literature therefore differ in type and size of apparatus, the substrate and flow conditions assessed, the tracers employed and the parameters explored (Williams, 1970; Muste, 2010). As such, custom flumes were constructed for this investigation to create ideal test conditions that enabled tracer studies to be conducted in replicated systems with identical physical and hydraulic characteristics. The aim was to create identical channels to enable the effect of specific variables (e.g. biofilm development) on hyporheic exchange to be investigated.

2.2.1 Experimental Rig

The experimental system consisted of 9 recirculating rectangular flume channels (0.01 m thick glass; 2.36 m long, 0.1 m wide), divided into 3 separate block units comprising of three flume channels. The flumes were artificial water channels that acted as a gravity chute for recirculated water, powered by a pump, through a pipe system as shown in Figure 2.1. To limit experimental variability the mean bed height, mean water depth and pipe lengths were fixed.

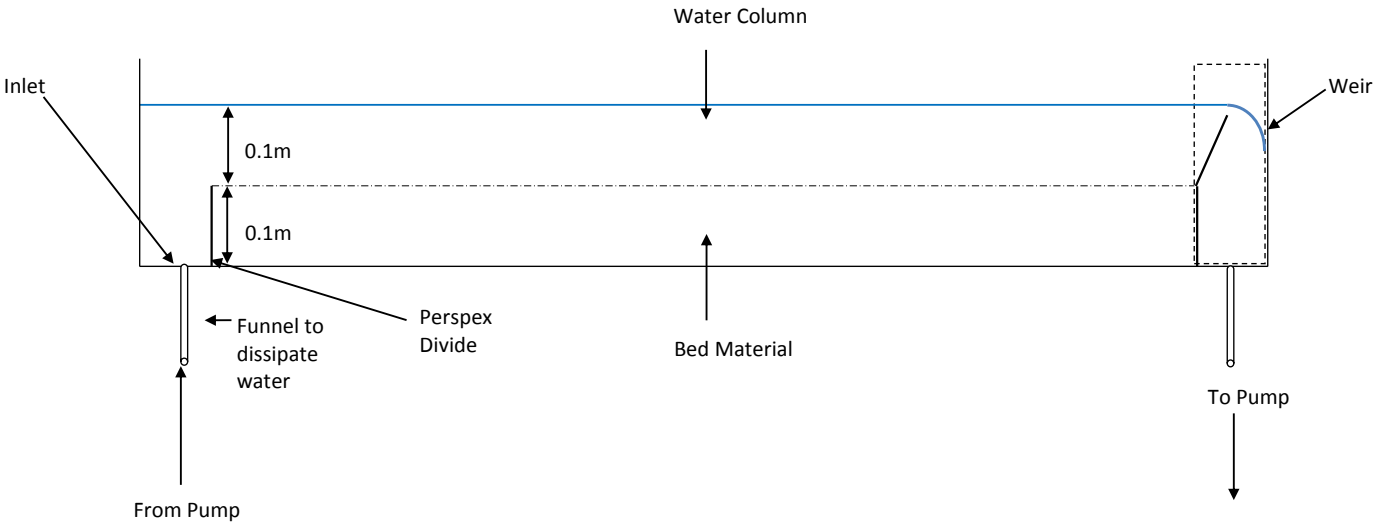


Figure 2.1a: 2D Illustration of the Recirculating Flume Channel

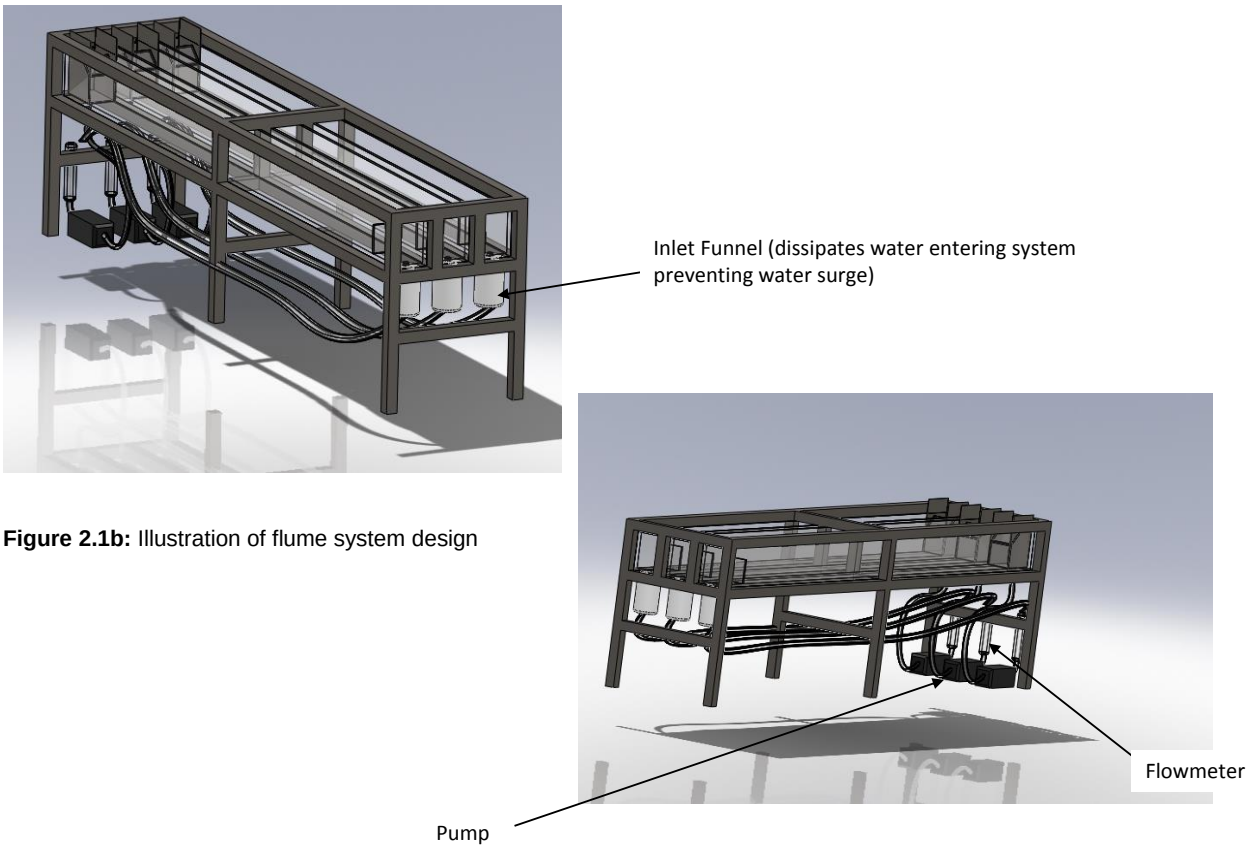


Figure 2.1b: Illustration of flume system design

2.2.1.1 Materials

The flume channels were completely made of glass to allow for clear visual observation of the experiment, and to enable easy deployment of optical instruments (e.g. Laser Doppler Velocimetry). The glass also acted as a smooth impermeable surface that could be easily cleaned and would not alter the water chemistry during prolonged testing. To maintain the structural rigidity of the system whilst maintaining pre-determined slope parameters, an aluminium frame was constructed to hold the glass sheets in place. This frame had adjustable legs to permit slope alteration, which would aid in generating uniform flow along the channel length for different flow velocities, sediment materials and sediment particle sizes. The experimental rig utilised a pump and pipe system to recirculate water in the system, creating a continuous infinitely long channel.

2.2.1.2 Pump/Mechanical components of system

The slopes placed on the flumes created a gravity chute down which water could flow. To recirculate this water a pump system was needed to recycle the water along the length of the channel. Clarke re-circulating pumps used in previous exchange experimentation (Chandler, 2013) were used due to their availability, employment in previous studies and large range of discharges (2 l/min to 40 l/min). These pumps were situated at the downstream end of the system; water flowed over the weir (used to regulate flow depth) and down the pipe where the pump re-circulated it through a flow meter and down additional piping until it re-entered the flume channel (Figure 2.2).

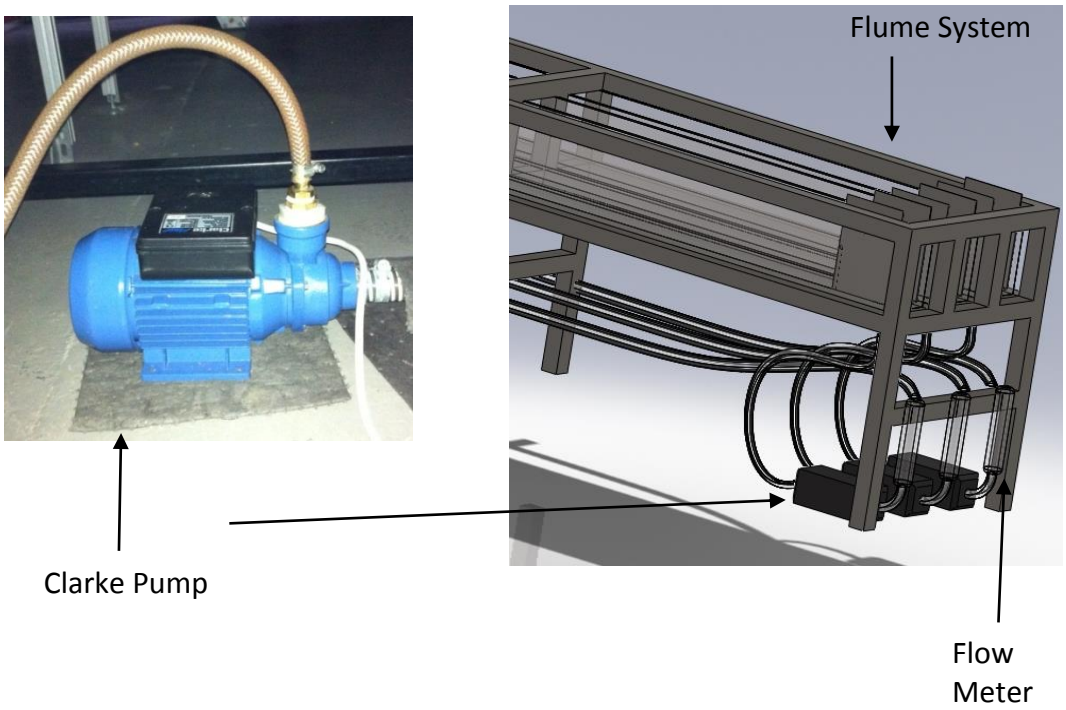


Figure 2.2: Initial Experimental Set Up with Clarke Pump

2.2.1.3 Sediment

Medium-fine quarry gravel (8.5 mm mean geometric particle size) and fine sand (0.5 mm mean geometric particle size) was initially used as the bed materials for testing the performance of the flumes. A 100 mm thick gravel bed was laid uniformly along the length of the channels, an example of which is shown in Figure 2.3.

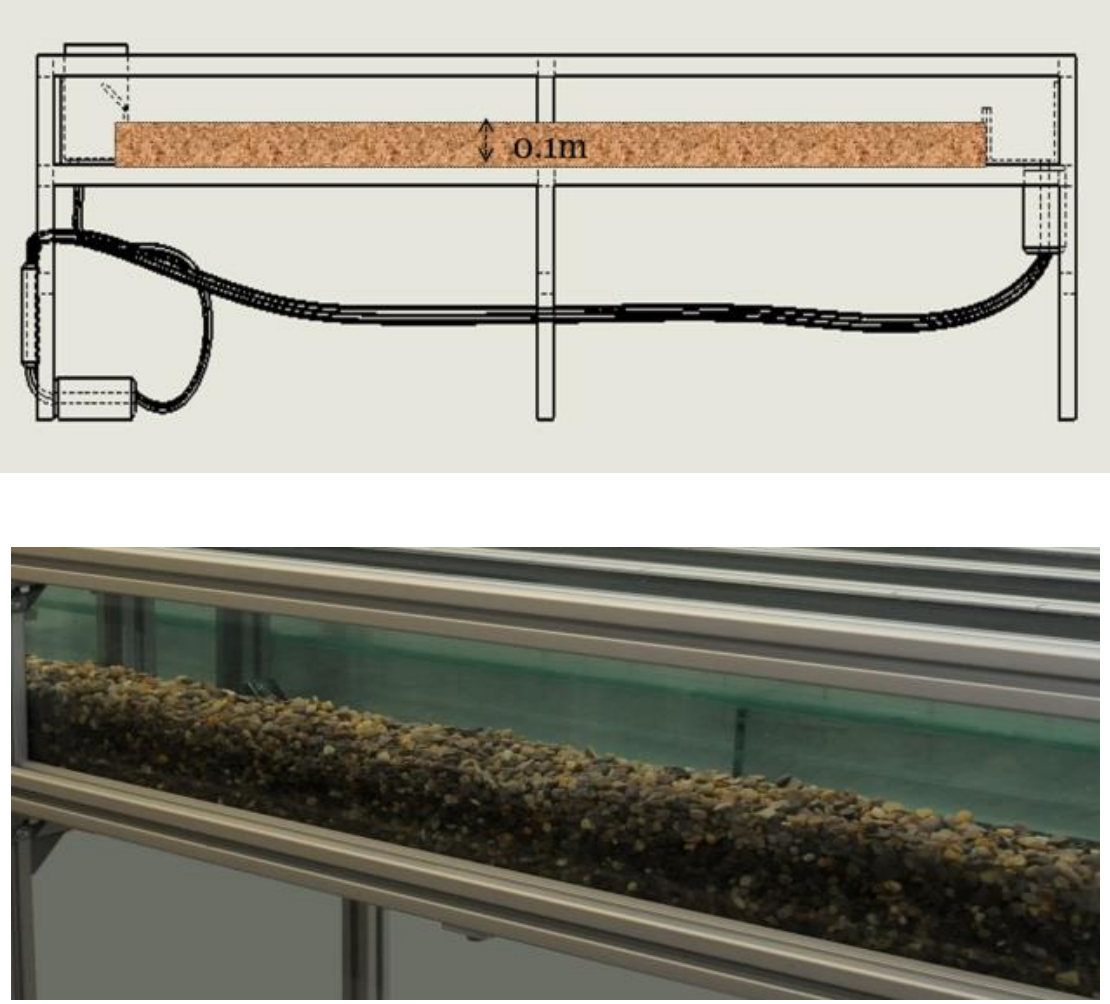


Figure 2.3: Uniformly laid 100 mm gravel bed *in situ*

2.2.1.4 Study Site

As the experimentation (Chapter 3) required the development of microbial communities, an inoculum source was required. To ensure that there was a sufficient abundance of microbes within the water employed in the flumes, water taken from the River Dene was used as an inoculum source.

The River Dene is a minor tributary of the River Avon, Warwickshire UK, and flows approximately 16 km from its origin in Burton Dasset Hills near Kineton to its confluence near Charlecote Park, illustrated in Figure 2.4. The water source is surrounded by a mixture of land use; nearby urban transport routes, residential and commercial areas, but is predominantly agricultural. The river has previously been used as a source of inoculum for chemical biodegradation tests (Kowalczyk, 2013) attributable to its identification as a “good’ to ‘very good’ biologically diverse unpolluted river (EA, 2009).

The River Dene has five wastewater treatment plants (WWTPs) along its water course and three WWTPs on tributary streams that flow into it. The contrasting size, influxes, treatment strategies and various other treatments parameters employed at each WWTP results in varying chemical and nutrient influxes into the water course, potentially generating variance in the biological community.

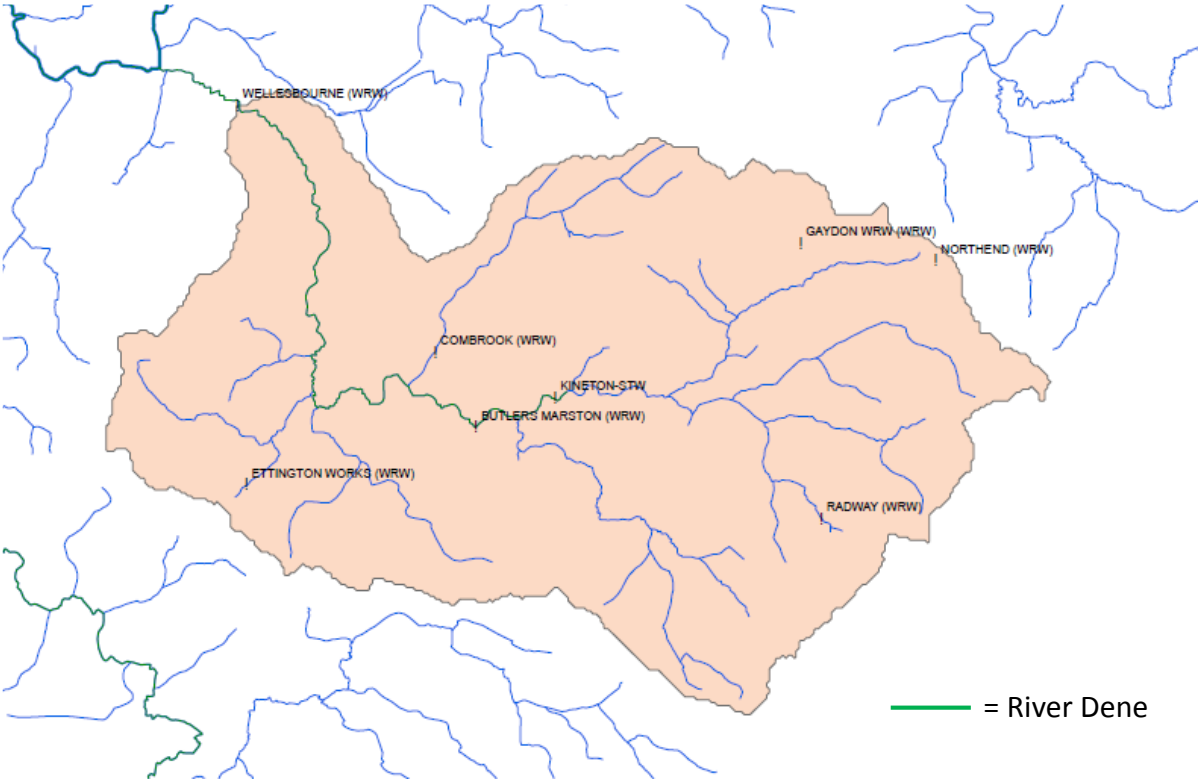


Figure 2.4: River Dene catchment showing distribution of Wastewater Treatment Plants along the watercourse.

Source: Williams, R., Centre for Ecology and Hydrology, UK.

River water was collected from one point source location prior to each flume test and and before use was passed through a 200 µm mesh to remove large particulate matter in the water (in keeping with OECD test 308 parameters). The source location was Wellesbourne, upstream of the largest WWTP in the River Dene catchment area, due to its accessibility and use in previous studies (Kowalczyk, 2013). The physiochemical attributes of the water were logged following collection to permit comparison between treatments (pH, water temperature, conductivity, PO_4 levels, NO_3 levels, dissolved oxygen levels).

Following collection, the river water was transported back to the lab and added to each flume. Uniform flow was then established.

2.2.1.5 Uniform Flow

Before each test uniform flow was established to ensure comparability in the channel. Flow is uniform if flow depth is constant along the channel, maintaining unvarying water area, velocity and discharge along the reach, guaranteeing that the water depth and channel surface are parallel (Chow, 1959), as seen in Figure 2.5. Uniform flow is rarely seen in natural environments, resulting in varied flow conditions (Chanson, 2004). However, results gained from uniform flow experiments create universal trend results as gravitational forces generated by the flow along the channel slope are in equilibrium with the resistance forces, exposing the entire channel to the same conditions. Additionally, uniform flow experiments produce an experimental study with fully defined parameters, making it more comparable.

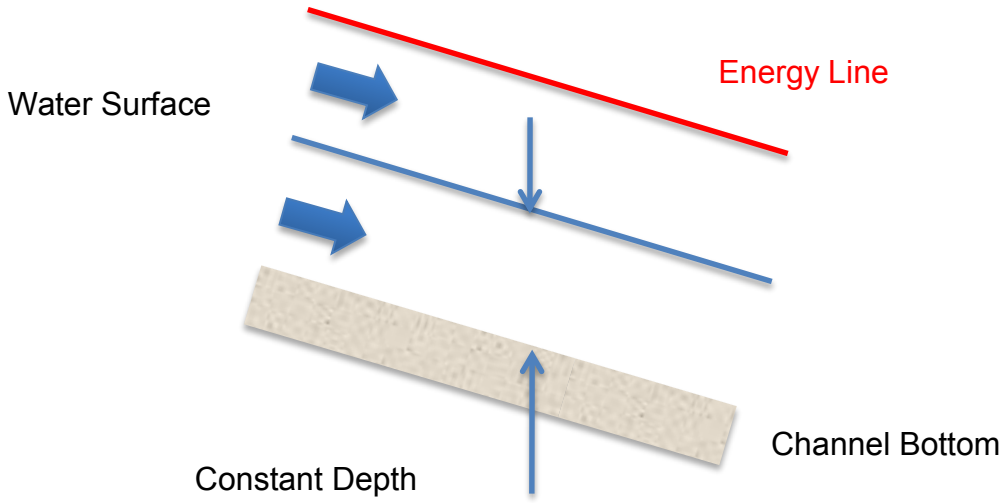


Figure 2.5: Establishment of uniform flow (energy line, water surface and channel bottom are all parallel)

Uniform flow was established using Vernier depth gauges across the length of the channel. The bed height and water depth were measured in relation to the flume base at two points across the width every 0.1 m along the length of the flume. To alter the flow depth the weir was adjusted accordingly and the base level of the flume adjusted until Uniform flow was achieved.

2.2.2 Fluorometry

To assess penetration of solutes at the water-sediment interface, fluorescent dye trace experiments have traditionally been undertaken (Smart and Laidlaw, 1977). In this experimental system trace experiments were conducted on bed-forms with different sediment parameters to assess the impact of biofilm development on the rate of exchange between the water column and the interstitial pore spaces in the sediment bed (Chapter 3). This is comparable to methodologies employed in other flume studies using tracers (Elliot and Brookes, 1997; Marion et al., 2002).

2.2.2.1 Tracer Selection

To assess the rate of exchange between the water column and the interstitial pore spaces in the sediment bed the fluorescent tracer Rhodamine WT was used. Rhodamine WT is a water-soluble fluorescent dye that is pink in colour and specifically designed for use as a tracer chemical (Smart and Laidlaw, 1977).

There are numerous tracers that could have been employed in experimentation; however Rhodamine WT was specifically designed to be highly detectable and exhibits low levels of photochemical decay (Smart and Laidlaw, 1977). These factors permit accurate testing with low concentrations of the tracer chemical over long durations with low background ‘noise’ in the system.

2.2.2.2 Instrumentation

During initial testing Turner Designs Cyclops 7 fluorometers were used to measure tracer concentrations (Figure 2.6). The Cyclops' emitted a green LED that excited the fluorophores within the Rhodamine WT due to the change in light wavelength surpassing 550 nm (Boxall, 2003; Smart and Laidlaw, 1977). The intensity of the emitted light was dependent on the concentration of the fluorescent tracer and could be used to calculate the concentration.

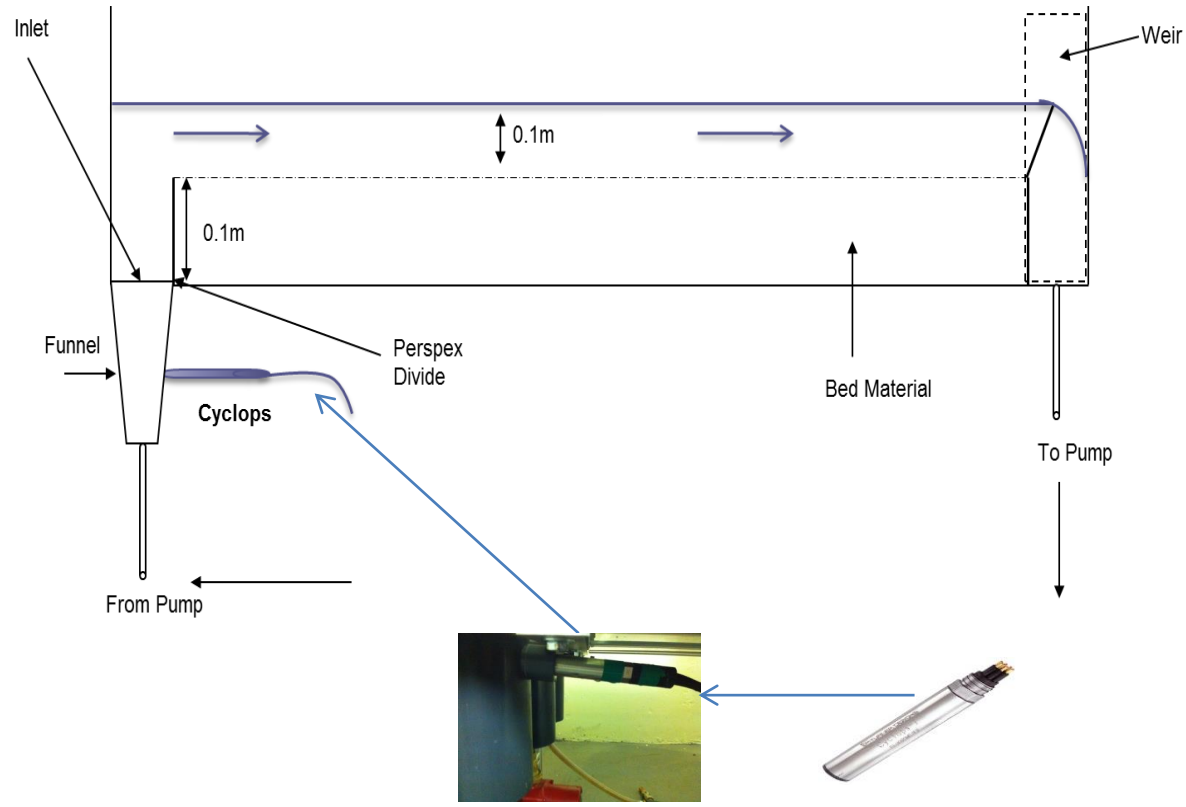


Figure 2.6: Turner Designs Cyclops 7 Fluorometer location

To measure the intensity of the emitted light, the instruments were placed in the inlet of each flume channel where water is re-circulated into the main water column. They were situated there to minimise the possibility of air bubbles transported in the water column settling on the instrument head and affecting the output. Situating the Cyclops fluorometers in the inlet meant that the decrease in fluorophore levels in the water column (as hyporheic exchange with the interstitial pore spaces in the sediment bed occurred) could be accurately

monitored. The light intensity was measured as a voltage using a photomultiplier. The equipment was calibrated *in-situ* using a concentration gradient (reading range from no light source to maximum plateaued light levels) to provide reference fluorescence values. An example calibration profile for the Cyclops fluorometers can be seen in Figure 2.7. The instruments record light levels emitted by the fluorophore in the water column and use this information to calculate resultant fluorescence values, from which a voltage is output as an analogue signal from the instruments which is then fed via coaxial cables to a Turner Designs data logger. This analogue signal is then converted to digital format and stored in the attached computer. The Cyclops log rates were controlled on the computer and fixed at a rate of 1 Hz and stored in a data file for viewing and analysis.

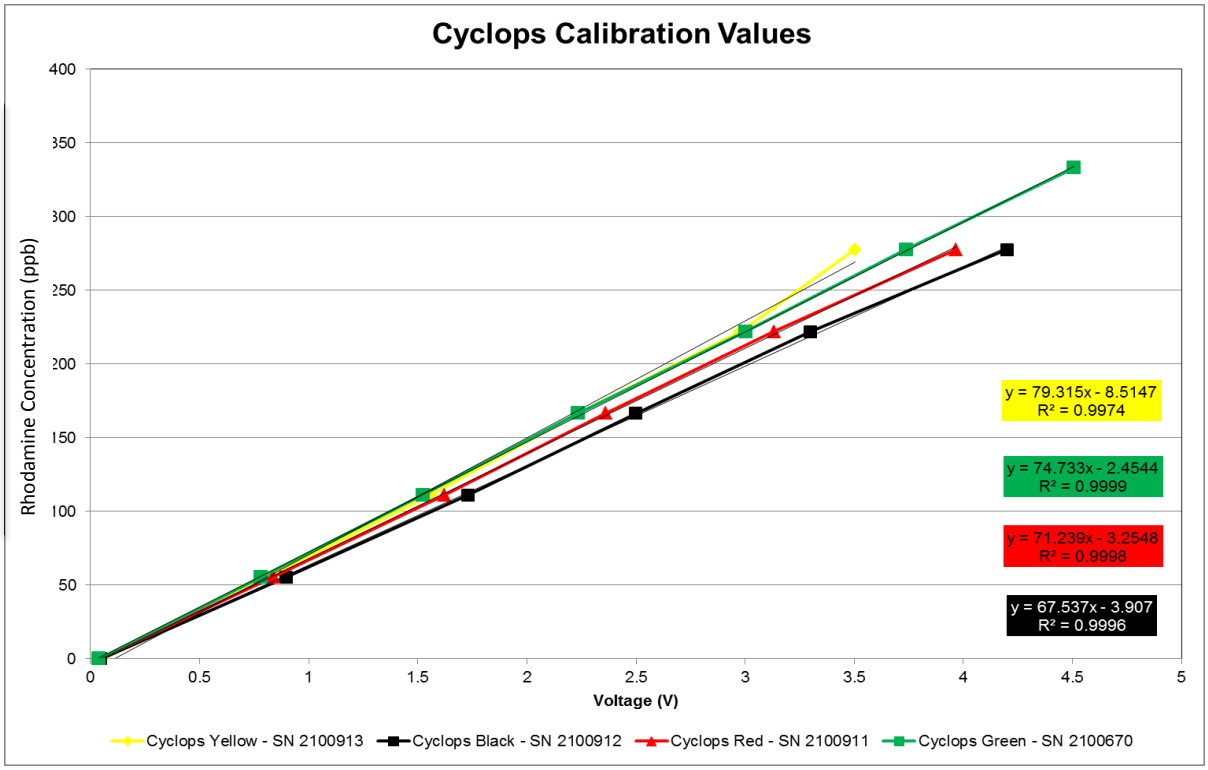


Figure 2.7: Example calibration profile for Turner Designs Cyclops 7 Fluorometers (*in situ* calibration: 09.12.2011)

The fluorometers were connected directly to a computer, which recorded the fluorescence levels at a rate of 100 Hz intervals over the course of the test. In addition to this, temperature probes logged the water temperature in each experimental flume system and in the room in which the flumes were housed, recording all information onto the computer logger. The temperature sensors used in this study were Tempatron NTC thermistors, which were enclosed in a stainless steel sheath (6 mm diameter and 50 mm long). The effective temperature scale of the sensor was −50°C to +110°C. The sensor was connected to a Wheatstone bridge, illustrated in Figure 2.8, and run from a 12V DC power supply. The temperature sensors were calibrated in a Grant JB2 water bath and set up so that the voltage was measured across the bridge and then related to the temperature based on the calibrations. During the course of the test, the flume channels were covered with cling film to inhibit loss of water due to evaporation.

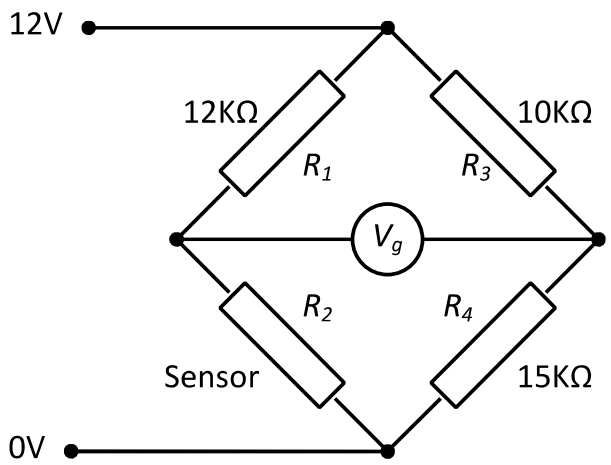


Figure 2.8: Wheatstone bridge used with temperature sensor

2.2.3 Experimental Development

To complete the primary objective of establishing an experimental system capable of analysing hyporheic exchange, experimental testing and modification was required. The experimental system required modification to eliminate oscillations, reduce temperature, identify the cause of tracer decay during testing, developing a dosing method and analysis of data.

2.2.3.1 Temperature

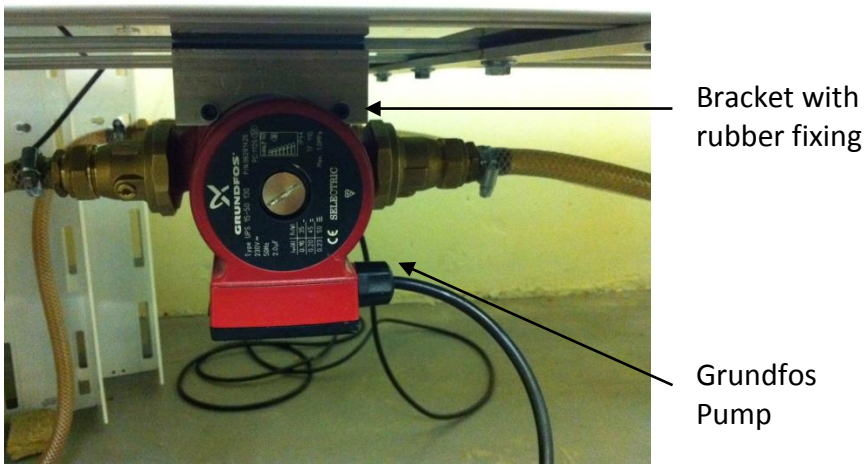
Rhodamine WT is a stable and suitable tracer for use in flume experimentation. However, its fluorescence levels have been identified as temperature dependent (Smart and Laidlaw, 1977). As such, experimental modifications were undertaken to account for temperature fluctuations.

2.2.3.2 High temperature re-circulation

As Rhodamine WT is temperature sensitive an optimum experimental system required constant steady temperatures. The initial experimental design used Clarke pumps, which were situated on the floor, re-circulating water up to a maximum discharge of 40 l/min (Figure 2.3). These pumps were able to produce a range of constant discharges. However, they caused the temperature of the water to rise from starting temperature of 18 ^{o}C to over 45^{o}C. These temperatures are not consistent with those of natural flowing water channels and cause inconsistencies in the recorded fluorescence levels.

To resolve this issue Clarke pumps (which recirculated water at lower discharges) were replaced with Grundfos UPS15 pumps. The experimental system was modified by side-mounting the pumps onto the rig to reduce the possibility of air pockets within the system (Figure 2.9). These pumps were capable of producing a range of discharges; however maximum discharge used

in the system was 12 l/m. Test runs indicated that the maximum water temperature in the system was 24 °C, with the average run temperature being 23.8 °C; more representative of UK river channel temperatures.



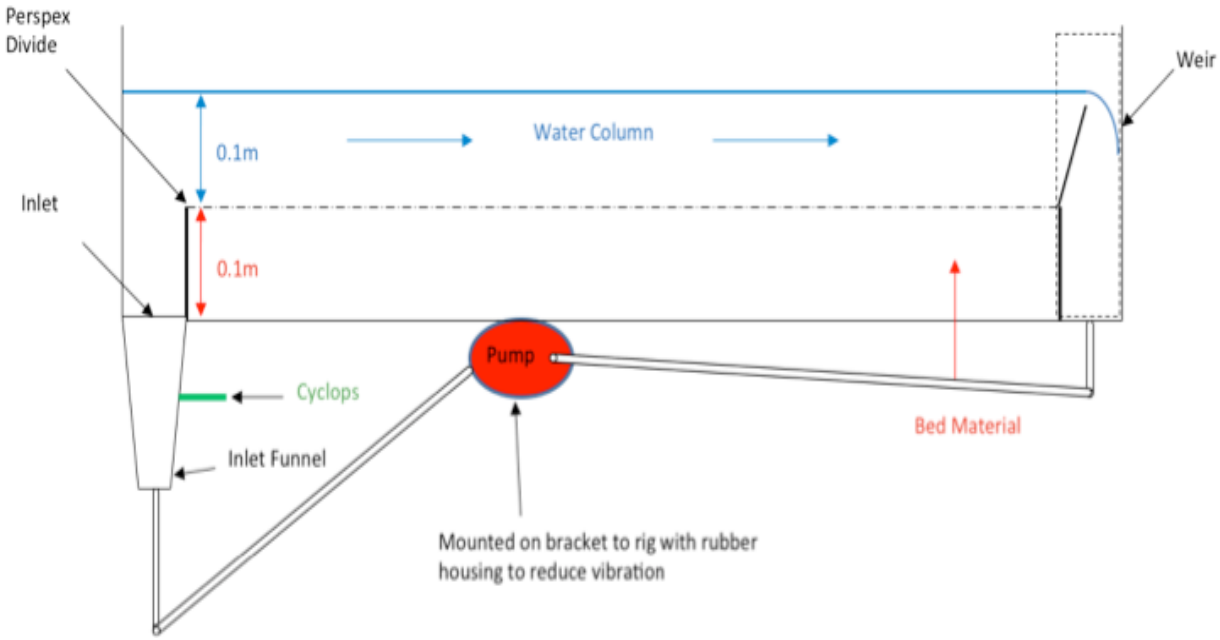


Figure 2.9: Grundfos pump Set Up

2.2.3.3 Temperature Compensation

Once the systems were built they were tested to assess reproducibility of parameters and assure that mass balance could be achieved within the flume system following addition of Rhodamine WT. Conducting re-circulating water tests to monitor the fluorescence levels over time following the addition of a tracer did this. These trace tests used Cyclops fluorometers and temperature loggers in each channel to directly relate temperature to concentration change. It was expected that an equilibrium concentration would be reached in the system over time, based on the amount of tracer added.

To test this, empty flume channels were filled with water and injected with Rhodamine dye to obtain an equilibrium concentration of 200 ppb in each channel. The concentration of Rhodamine and the temperature was logged as described in Section 2.2.2.2. These channels were each filled with 50 litres of water and re-circulated at 12 l/min. They were injected with 10 ml of Rhodamine WT to give an expected end concentration of 200 ppb. The test ran for 48 hours to identify if there was any fluctuation in recorded fluorescence levels in response to temperature variation. The recorded temperature levels were then directly compared to fluorescence in order to establish whether temperature compensation algorithms were needed for long term testing.

2.2.4 Tracer Decay

To assess penetration of solutes at the water-sediment interface, fluorescent dye trace experiments were conducted on different sediments to establish whether mass balance could be achieved in the experimental systems in the presence of a porous bed material whilst assessing the impact sediment type has on the rate of exchange between the water column and the interstitial pore space in sediment bed (see Smart and Laidlaw, 1977). Tests were conducted on different bed profiles and on different sediment types; sand (150 µm) and gravel (7.5-10 mm). The bed profiles used remained the same in all tests,

irrespective of bed material, with each test maintaining a constant mean sediment height (10 cm), mean flow depth (3 cm), mean discharge (12 l/min) and bed slope (changed for each material, bed-form and discharge).

The test involved injecting a Rhodamine tracer into the re-circulating flow at the weir end of the flume. After the addition of tracer to the system, the fluorescence levels were logged over time. It was expected that an equilibrium end concentration level would be reached in the system based on the amount of tracer added once complete mixing within the system had occurred.

To ensure whether any reduction in recorded fluorescence levels was attributable to retention in sediment, further tests were conducted. An experiment was set up to identify whether Rhodamine could be retrieved from the sediment. Immediately following the completion of the trace test the water was drained from the channel and replaced with clean, deionised water. As Rhodamine is water-soluble no chemicals were required to remove the Rhodamine from the sediment. The water was left to recirculate over the sediment bed for 3 days and fluorescence monitored to establish whether the sediment had absorbed any of the Rhodamine tracers in the previous trace study, thereby accounting for any potential loss in Rhodamine during the original test.

A further test series was conducted to identify whether replacing the natural sediment bed with a synthetic, uniform substrate of glass beads eliminated tracer decay (Test parameters are shown in Table 2.1). This test was set up in the same way as described in Section 2.2.2.2 but employed synthetic glass beads as an artificial substrate instead of natural sediments.

Table 2.1: Glass bead Flume Test Experimental Parameters

Parameter	**Glass Bead Bed**
Discharge [Q] (l/min)	12
Mean Velocity (m/S) (Q / [Channel Width x Flow Depth])	0.057
Flume Number	8
Bed - Form	Flat
Bed Material	Glass Beads
Mean bed height (m)	0.1
Mean channel depth (m)	0.035
Volume of Water in flume (l)	18.1
Calculated Reynolds Number	4688
Volume of Rhodamine WT solution (1×10^6 PPB) added with each repeat discrete injection (l)	0.002
Expected End Concentration increase with each consecutive dose (PPB)	112
Turner Designs Cyclops 7 Fluorometers Serial Numbers	2100913

2.2.5 Consecutive Dosing

Longer-term experiments required a testing methodology that incorporated consecutive dosing to assess variations in exchange over time due to biofilm development in the flume system. To ensure such comparisons were repeatable and comparable, consistent dosing was essential. As such, the comparability of continual tracer dosing was compared in the flume system with a raised glass sheet acting as a fixed impermeable bed. This experimental design would allow the immediate mixing within the water column and the added interaction of hyporheic exchange into the sediment bed to be determined, thereby identifying the tracer injection time-frame for complete mixing within the water column. Continual dosing of Rhodamine was conducted in a flume channel at four separate time intervals to achieve end concentration increments of 80 ppb to compare the mixing rates in the water column, establishing whether time point injection affected mixing rates. The experimental design described in Section 2.2.2.2 was followed with the variation of not using a substrate to remove hyporheic exchange as a variable, conducted in conjunction with experiments described in Section 2.3.2.

2.3.4 Sinusoidal oscillations

Testing progression was impeded due to cyclic sinusoidal oscillations recorded on all raw data from the Turner Designs Cyclops 7 submersible fluorometers, which were used to monitor change in fluorescence concentration in the water column (Figure 2.10). This was a problem that had not been seen in other experimentation using these instruments (Chandler, 2013). Time was taken to re-evaluate the experimental system as the presence of the oscillations affected the data readings.

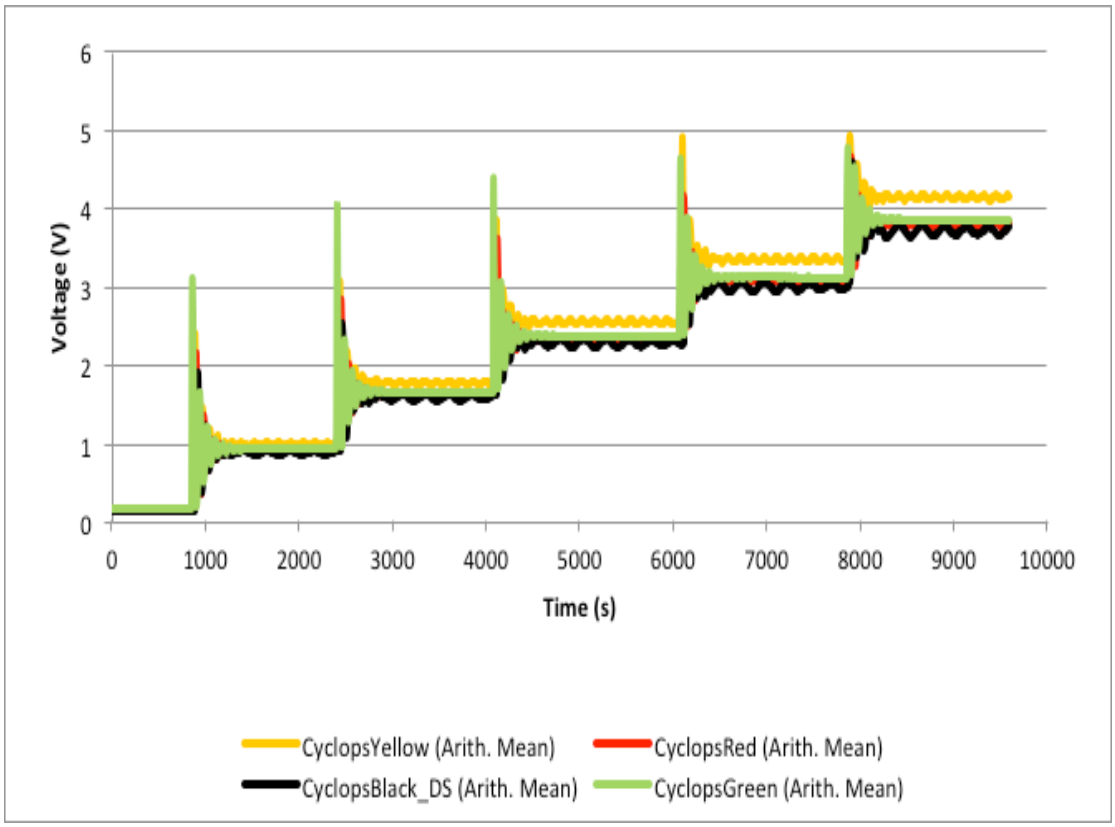
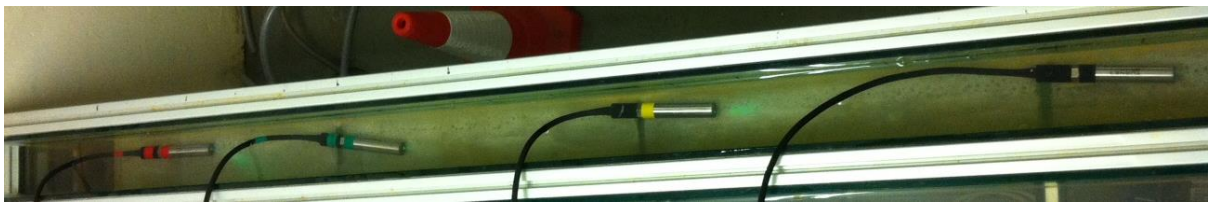


Figure 2.10: Example of oscillations recorded during an *in situ* calibration of the Cyclops fluorometers in a re-circulating flume channel

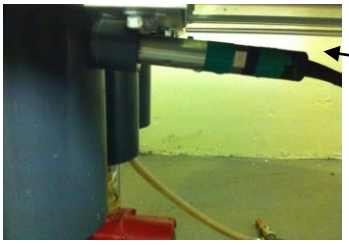
The presence of these oscillations required collaborative experimentation with the suppliers and manufacturers (RS Aqua and Turner Designs) in an attempt to rule out all possible peripheral circumstances that could be attributable for the fluctuations. Testing focused on amending the experimental set up to rule out all aspects of the system as the causal factor.

Systematic assessment of the system tested every individual component individually. After each variable in the experimental unit had been assessed the oscillations were still present. Further testing was then conducted in which all instruments were situated on the glass bed of one of the flumes whilst the water was re-circulating when logging commenced (Figure 2.11). Instrumentation was then systematically removed from the flume to a separate re-circulating flume channels to see if independent placement of instruments would resolve the issue.



Instruments Submerged in a single re-circulating flume

Instruments moved into different channels to identify impact on oscillation



Instruments moved into inlet of channel to identify impact on oscillation

Figure 2.11: Testing progressive removal of Cyclops Heads from one channel to another to assess impact on oscillation presence

These tests identified that whether the presence of multiple Cyclops heads in the water column possessed an impact on the occurrence of the sinusoidal oscillations recorded in the raw data. Isolation of the photodiode on the instrument using an 'end cap' was then undertaken to confirm that separation of the instruments removed the oscillation (Figure 2.12). This would confirm whether the causal factor of the sinusoidal oscillations was multiple instrumentation interference.

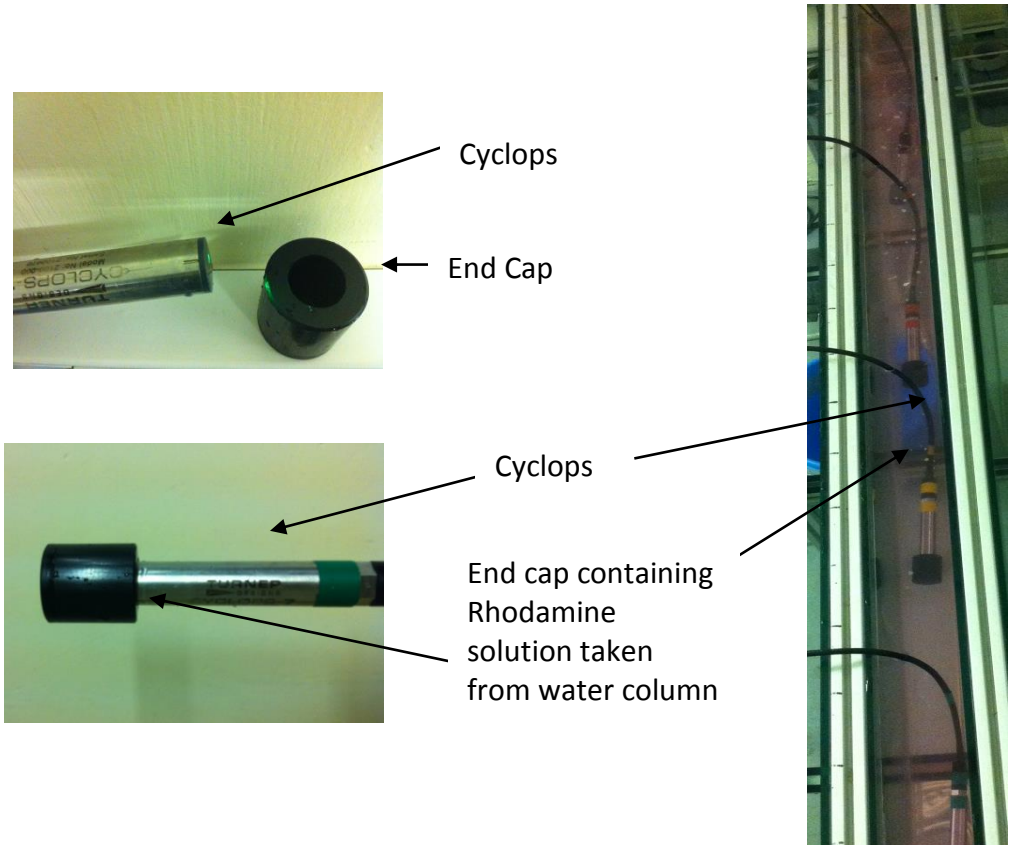


Figure 2.12: Experimental set up for light feedback test using black plastic caps to enclose the LED of the instrument in Rhodamine solution from the surrounding water column to identify if separation of instruments removed oscillations

2.2.7 Flow Depth

To maintain uniformity provisional tests had a fixed bed height and flow depth of 0.1 m, creating a 1:1 sediment:water ratio. However, when preliminary trace tests were conducted the rate of exchange of the injected dye from the water column into the interstitial pore spaces in the sediment bed was extremely slow. As such, calculations were conducted to assess the mixing regime in the system. Flow regime was ruled out as the mass transfer limiting step.

At 0.1 m flow depth the mixing was found to be poor/slow, whereby the fluids were flowing slowly, in discrete layers, with limited mixing between them (Rutherford, 1994). As this type of flow would not provide useful data for hyporheic exchange experiments the flow depth was lowered to 0.03m, where the Reynolds number would surpass 5000 and the flow conditions would be turbulent. This provided a more suitable test flow, as the water column would exchange with the sediment bed.

2.3 Functional Schematic

Following the provisional work conducted to establish a reproducible experimental system, modifications had to be made to enhance the effectiveness of the experimental design.

2.3.1 Defined Parameters

The experimental rig was altered to allow the water recirculate at a lower temperature; changing the pump permitted this (Section 2.3.1.1). The flow depth was lowered to ensure turbulent flow occurred in the system (Section 2.2.2.2) and a single Cyclops fluorometer was used in the inlet of each system. The modified experimental rig is illustrated in Figure 2.13.

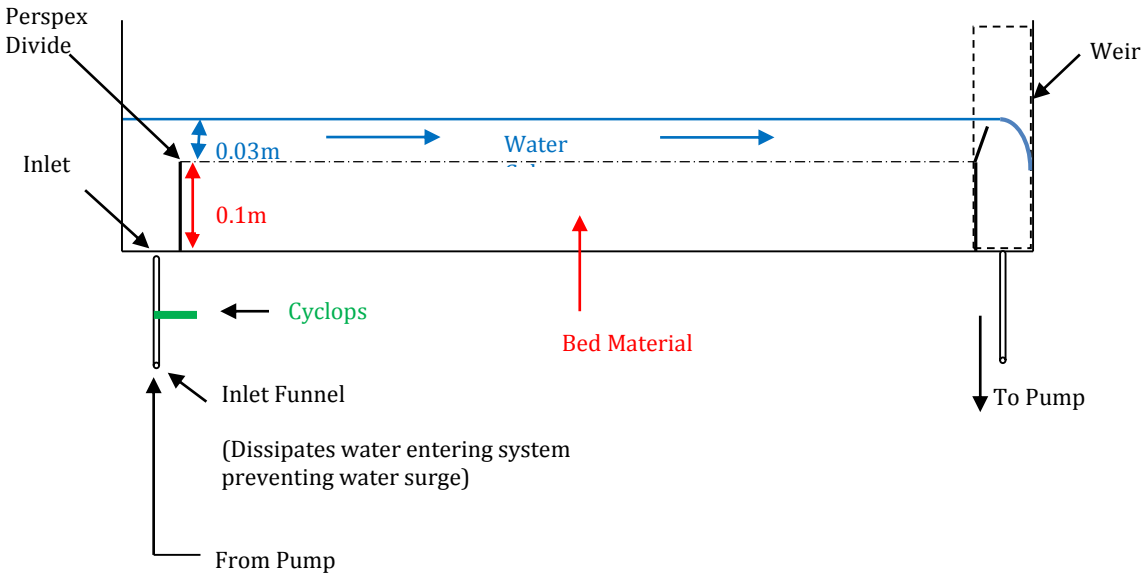


Figure 2.13: Flume System

2.3.2 Example Trace Test

With the modified system, trace testing could commence. Figure 2.14 illustrates an example output trace that logged the hyporheic exchange of as Rhodamine WT injected into the water column mixed with the interstitial spaces in the porous sediment bed. The figure shows the initial spike in recorded fluorescence levels following tracer injection, then the gradual decline of its concentration in the water column as the dye mixed within the system until equilibrium was reached; shown by the eventual plateau at the expected end concentration (112 ppb).

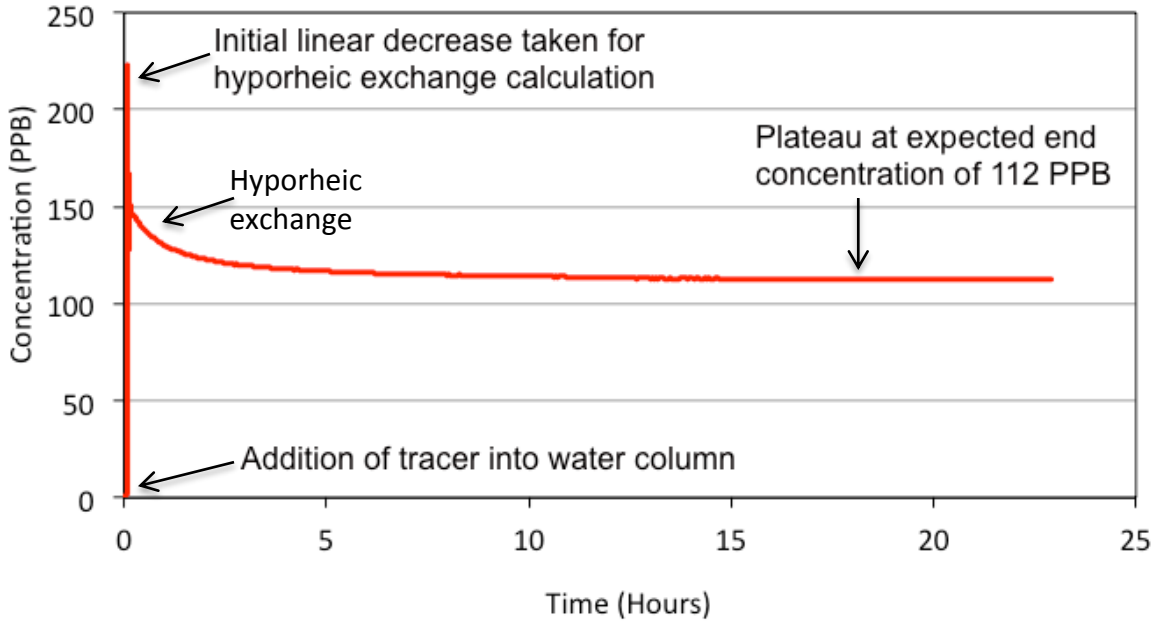


Figure 2.14: Example trace to illustrate recorded exchange in experimental rig (Q = 12 l/min; Bed = Glass Bead; Bed-Form = Flat; Flow Depth = 0.03 m; Expected end concentration = 112 ppb; Recorded end concentration = 111.8 ppb; Date = 11/11/2012)

2.3.3 Analytical Development

To quantify variance in rates of hyporheic exchange the trace data generated required further analysis. A plethora of methods have been proposed for accurate modelling of hyporheic exchange (see Packman and Bencala, 2000). However, these methods do not take flow variations or alterations in sediment characteristics into account, resulting in disparities between models and recorded data. As such, an accurate analytical methodology was required before progression to full scale testing could commence.

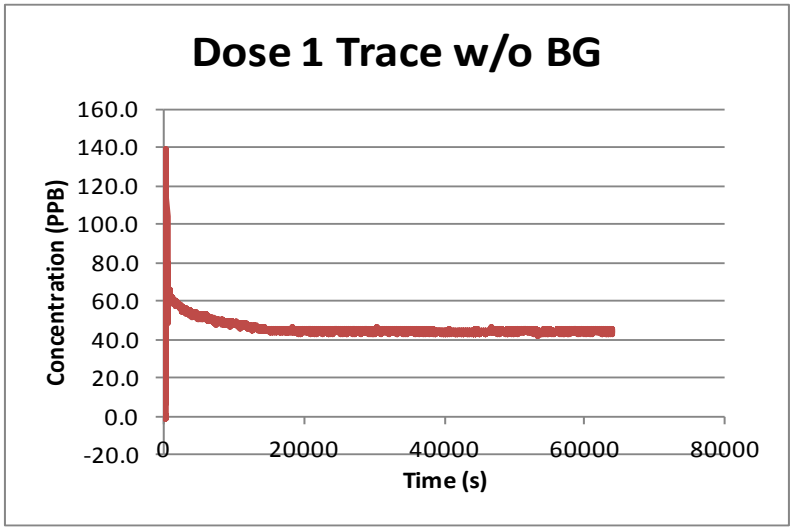
2.3.3.1 Established analysis

The work of O'Connor and Harvey (2008) collated data from hyporheic exchange flume experiments to create a data set encompassing a large spread of flow and sediment conditions. A measured effective diffusion coefficient was calculated for each study, requiring different calculations to be employed based on injection and sampling locations (i.e. bed or channel). O'Connor and Harvey (2008) specified a scaling constant and exponents by plotting three dimensionless numbers against the dimensionless diffusion coefficient, which demonstrated a strong correlation between the Péclet permeability number ($Pe_k^{6/5}$) and shear Reynolds number (Re). A Péclet number is defined as a dimensionless number that relates to the effectiveness of mass transport by advection to the effectiveness of mass transport by either dispersion or diffusion (Fetter 1999).The Reynolds number is a dimensionless value used in fluid mechanics to indicate whether fluid flow is steady or turbulent (Chanson, 2004). The work conducted produced an analytical method to quantify hyporheic exchange coefficients that matched the findings of the trace tests explored.

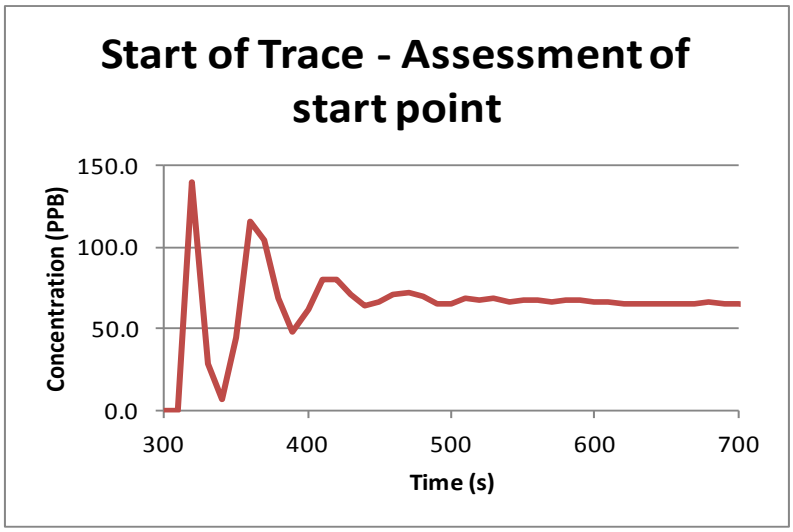
However, vagueness used in O'Connor and Harvey's (2008) described analytical method required in depth testing and sensitivity analysis. This was particularly the case, as subjective analysis was required to derive the "initial

exchange" parameter, the value or extent of which was not defined. This then questioned the robustness of this analytical methodology.

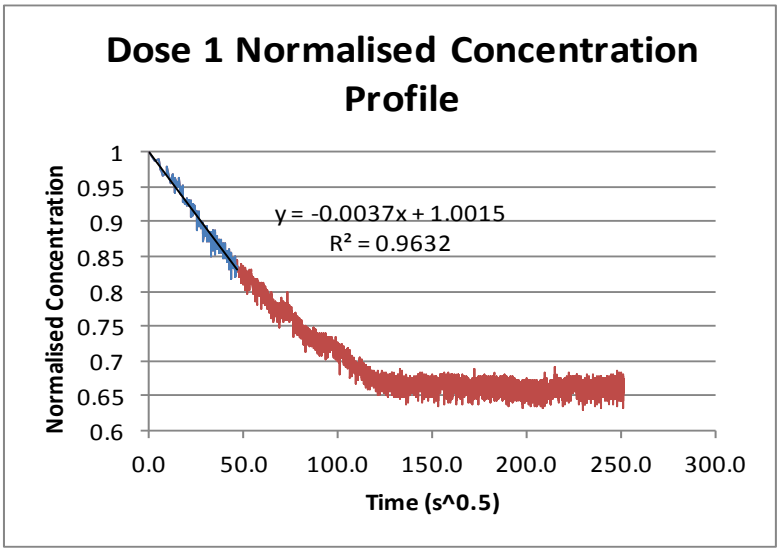
O'Connor and Harvey's (2008) hyporheic exchange coefficient required the normalisation of the trace data to ascertain the initial gradient of exchange. This was calculated by dividing all data points by the start value to remove all background deviation. As no definition was provided for this parameter, sensitivity testing was undertaken using previously published work (Marion *et al.*, 2002) to identify whether comparable exchange values could be produced to those generated by O'Connor and Harvey (2008) using their "vague" equation. The O'Connor and Harvey (2008) methodology employed is briefly described in Figure 2.15.



Convert voltage output to ppb using calibration equation and remove background



Identify start point of trace from which normalised concentration can be established



Calculate normalised concentration and establish gradient of 50% of the total change in normalised concentration during testing.

$$D_e = \left(\frac{\sqrt{\pi}}{2}\frac{d(M'/\theta)}{d(t^{1/2})}\right)^2$$

Figure 2.15: Analytical procedure used to establish Hyporheic Exchange gradient

Utilising 50% of the slope as the "initial gradient" in the calculation provided hyporheic exchange coefficients comparable to those generated by O'Connor and Harvey in their analysis, as shown in Figure 2.16. This suggests that the parameters used are suitable for employment in the analysis of hyporheic exchange values. It should be noted that, irrespective of the high correlation, there is a slight offset on the x-axis which is attributable to the use of an alternate equation when calculating shear stress velocity (u_*); this is because O'Connor and Harvey (2008) used $\sqrt{GHS}$ to ascertain a parameter of their calculation of $Re_*Pe_k^{6/5}$ values which is a non-conventional method that could not be replicated due to limited availability of their numeric variables. As such, the common convention of using $\sqrt{GRS_o}$ was employed, causing a slight offset on the x-axis.

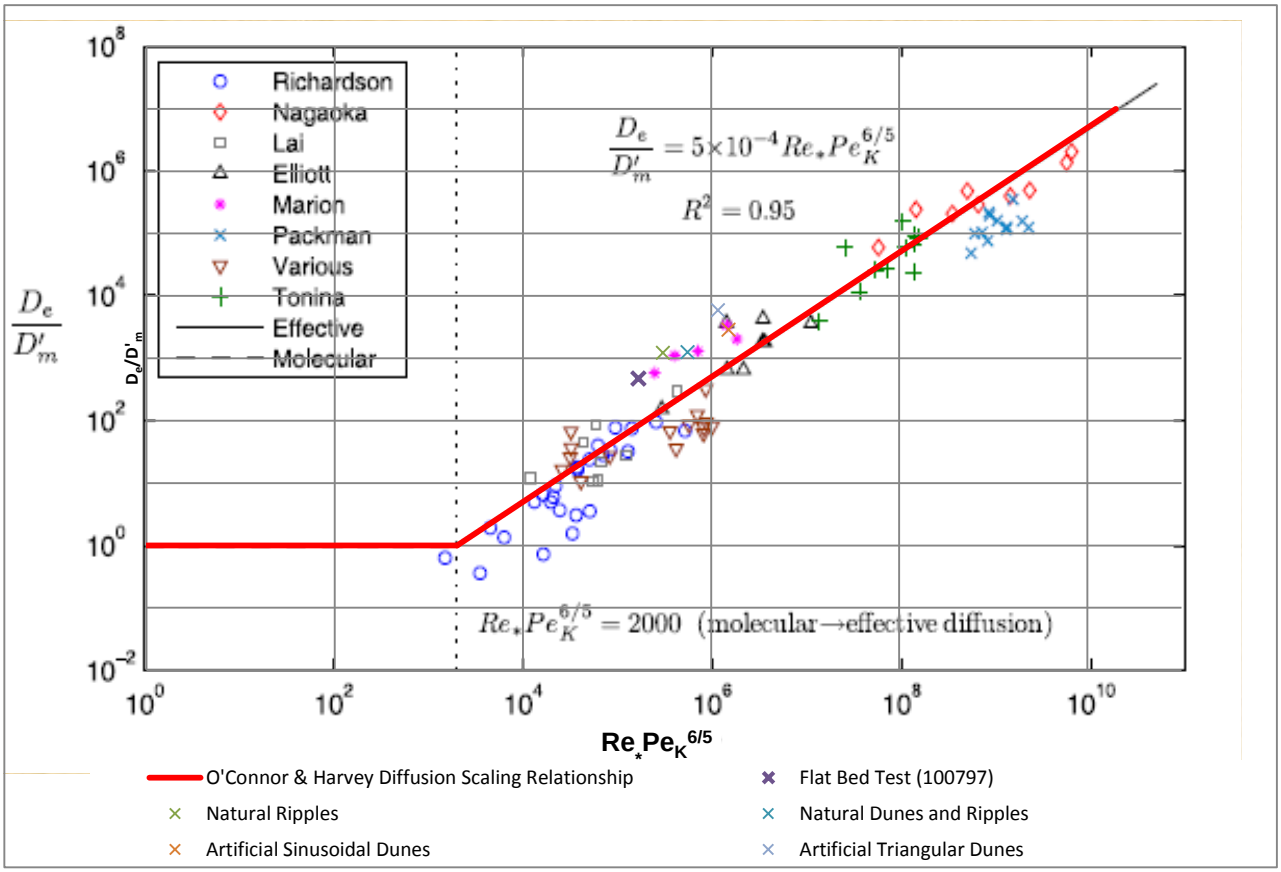


Figure 2.16: Obtained values of Marion *et al.* (2002) overlaid onto O'Connor and Harvey's values

2.3.3.2 Analysis using GenStat

The O'Connor and Harvey (2008) methodology had the potential to calculate the hyporheic exchange coefficients for the trace tests. However, the injection method employed in this study did not generate a clear start point for the trace data, meaning that any analysis conducted may have been deemed as qualitative due to the nature of manual start point selection. Additionally, the data-sampling rate impacted the analysis as logging conducted at 10-second intervals during the tests resulted in large amounts of scatter on the concentration profiles generated.

To eliminate inaccuracies, and remove the chance of error in the data analysis, further investigation was required to generate a standard reproducible method of start point identification from which comparison gradients could be quantified for utilisation in O'Connor and Harvey's (2008) hyporheic exchange calculation. To do this a computer programme and script was generated to conduct identical analysis on all datasets, eliminating subjectivity and human error.

2.3.3.2.1 Reduction of scatter: Smoothing

Following trace test completion the voltage data collected using Cyclops Fluorometers was converted to concentrations (ppb) using calibration equations and corrected for temperature. The raw data was collected at 10-second intervals which created excess scatter on the output plots, meaning that there was substantial noise on each individual trace. The presence of noise on traces possessed the potential to impact the accuracy of gradient calculations.

The data was input into General Statistics data analysis software (GenStat) for computational analysis. Primarily, the trace data was smoothed using application of a moving window average spanning 60 seconds to permit easier curve fitting and eliminate inaccuracies from the data analysis method. This 60-second moving window average was identified by sensitivity analysis. Figure 2.17 illustrates the difference seen between pre and post-smoothing data plots on a standard trace in the re-circulating flume system.

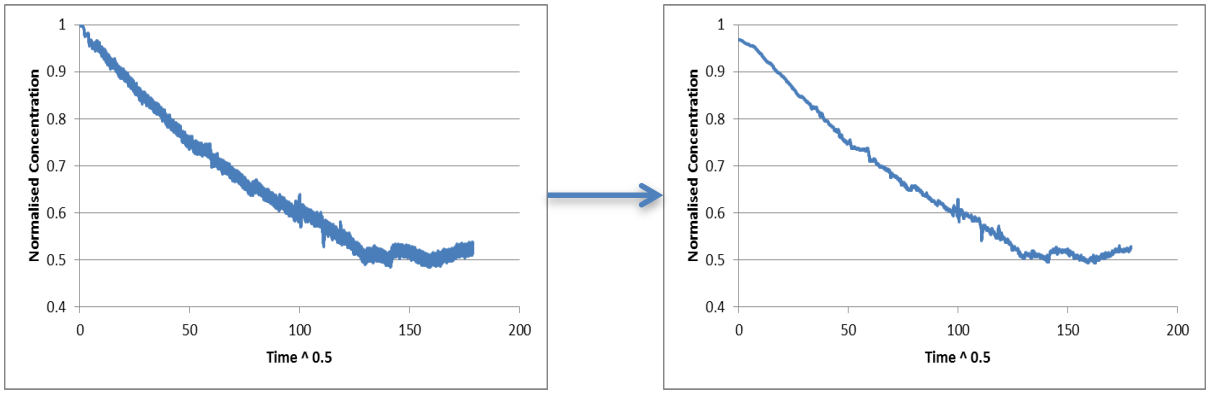


Figure 2.17: Application of smoothing to trace data extracted from 5 mm sediment trace test

2.3.3.2.2 Gradient Identification

Following smoothing the GenStat software identified the maximum value (M_v) for each trace and used this value to split the data into two subsets of the original dataset using a logical expression which divided the data into two subsets, fitted by the data points relationship with the time function of the M_v. This was done to generate a computational method of start point identification that was constant across all tests and eradicated human error, thus improving the reliability of the tests. This is illustrated in Figure 2.18.

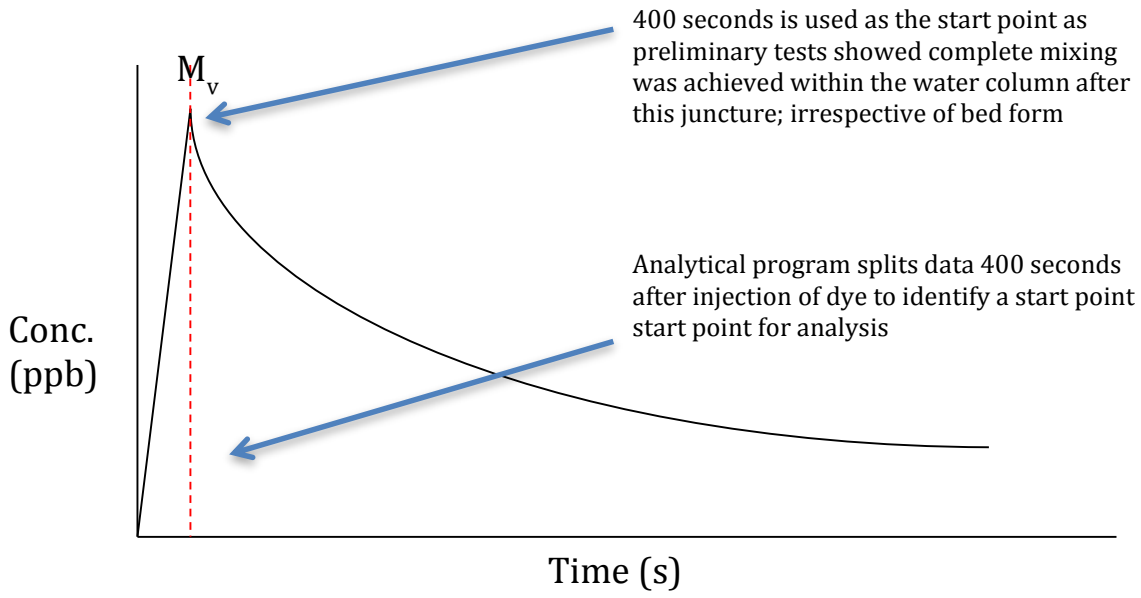


Figure 2.18: Decomposition of trace data for analysis providing an example of data analysis utilising GenStat

The first subsets of each trace, encompassing all the data points prior to M_v, were not focused on as they were not relevant to the exchange rates that the investigation examined. The M_v was used as the start point of each trace test. An exponential curve was then fitted to the data points after the M_v to determine the decrease in fluorescence concentration in the water column of each trace with time. However, an asymptotic value, based on the theoretical expected end concentration of each test, was added to the exponential curve to eliminate fluctuations at the end of the trace, stabilising the exponential curve at the end of the time period. The equation used for this curve (y) is:

$$y = (M_v - B) \cdot e^{-Rt} + B$$

Where M_v is the maximum value, B is the asymptotic value (which varies dependent on water volume in experimental system), R is the rate of exchange and t is time.

Following the application of the exponential curve, the 'initial gradient' was required to calculate the hyporheic exchange coefficient using the method described by O'Connor and Harvey (2008). As there were no pre-defined boundaries of the initial gradient, preliminary tests (conducted on raw data from Marion *et al.,* 2002) suggested that utilising 50% of the gradient of the exponential curve from the maximum value would generate results in keeping with those of O'Connor and Harvey (2008). As such, the GenStat programme output the gradient of the exponential curve at 50% (from the M_v) based on previously conducted sensitivity analysis, in order to calculate the hyporheic exchange coefficients.

This method of analysis identified gradients for each independent trace test and permitted *inter-* and *intra-* test series comparisons, from which reproducible quantitative analysis could be undertaken. Moreover, this analytical method could be modified to assess comparative gradients using different start points, such as the theoretical saturation point of the water column for each test, and output different predefined 'initial gradients.'

2.4 Results

2.4.1 Temperature Compensation

The trace tests conducted to establish the equilibrium stability of the system over time incorporating temperature variability is shown in Figure 2.19. These tests demonstrated decay in recorded concentration over time in the absence of any other variables, with the observed concentrations falling below the expected end concentration levels of 200 ppb. Further analysis showed direct comparison of recorded concentrations and temperatures from each flume channel displayed a positive correlation; temperature increase saw fluorescence level increase (Figure 2.20).

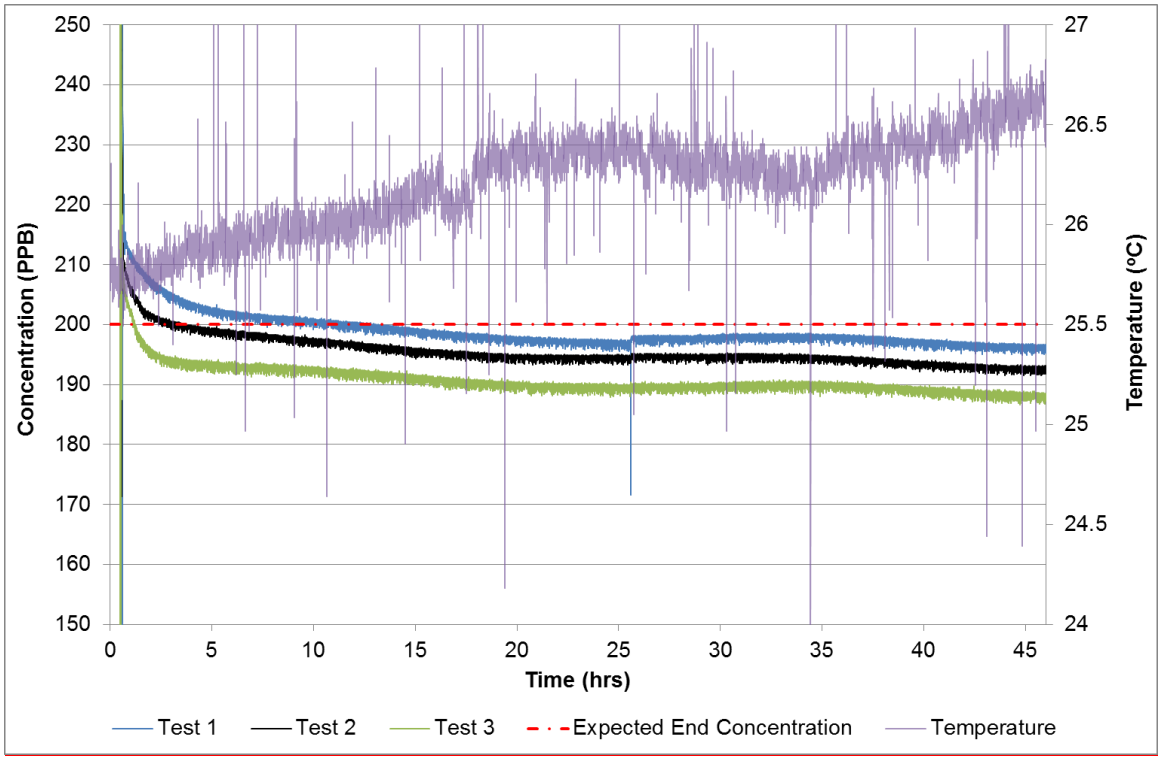


Figure 2.19: Dye trace conducting in 3 different recirculating flume channels

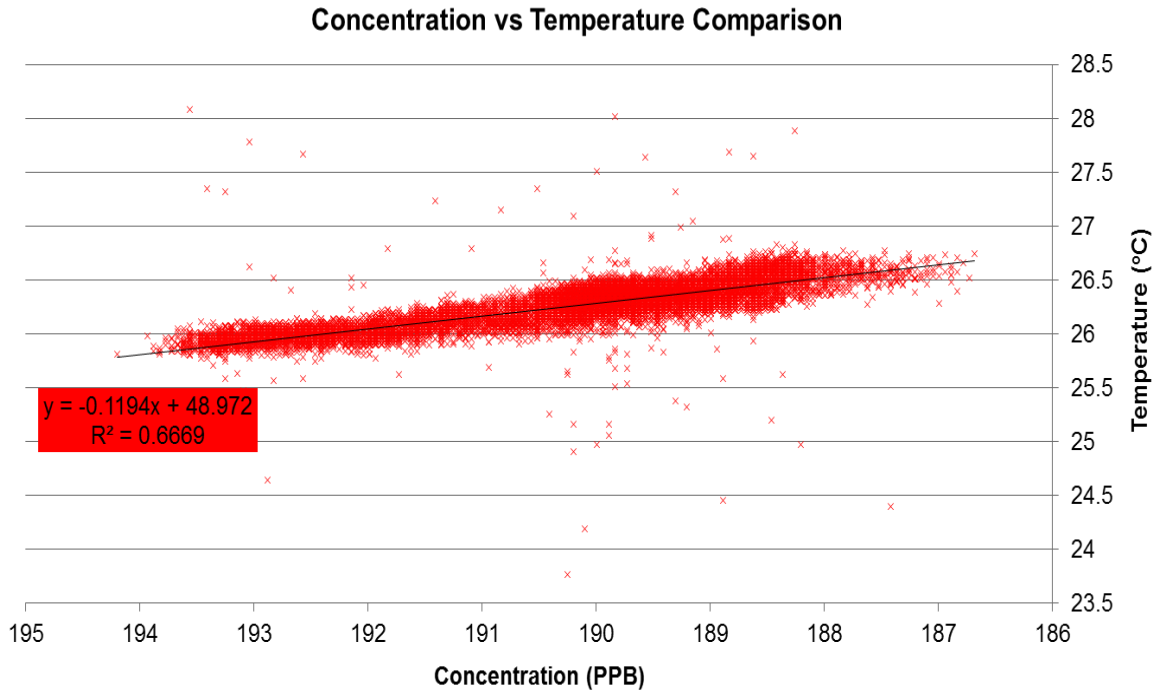


Figure 2.20: Relationship between the temperature and recorded Rhodamine concentration in Flume 3

This temperature variation can be recorded and applied to concentration data to compensate for temporal temperature fluctuations (Figure 2.21a). Figure 2.21b assesses the temporal difference between recorded concentration and final asymptotic value after the initial mixing phase to identify comparability of decay times in the different systems after temperature compensation. Figure 2.21b uses equations converted based on exponent multiplier to provide following decay constants, showing that Test 1 has a decay constant of 38 hours, which is comparable to that of Test 2 (40 hours). Conversely, there is larger a characteristic decay constant in Test 3 (61 hours), suggesting a slower rate of tracer decay in this test system. However, as the tests were stopped after 24 hours, this variance in decay rates is unlikely to impact the recorded exchange. Moreover, as no two river systems act in a comparable manner, it could be argued that such variance would be seen in the natural environment.

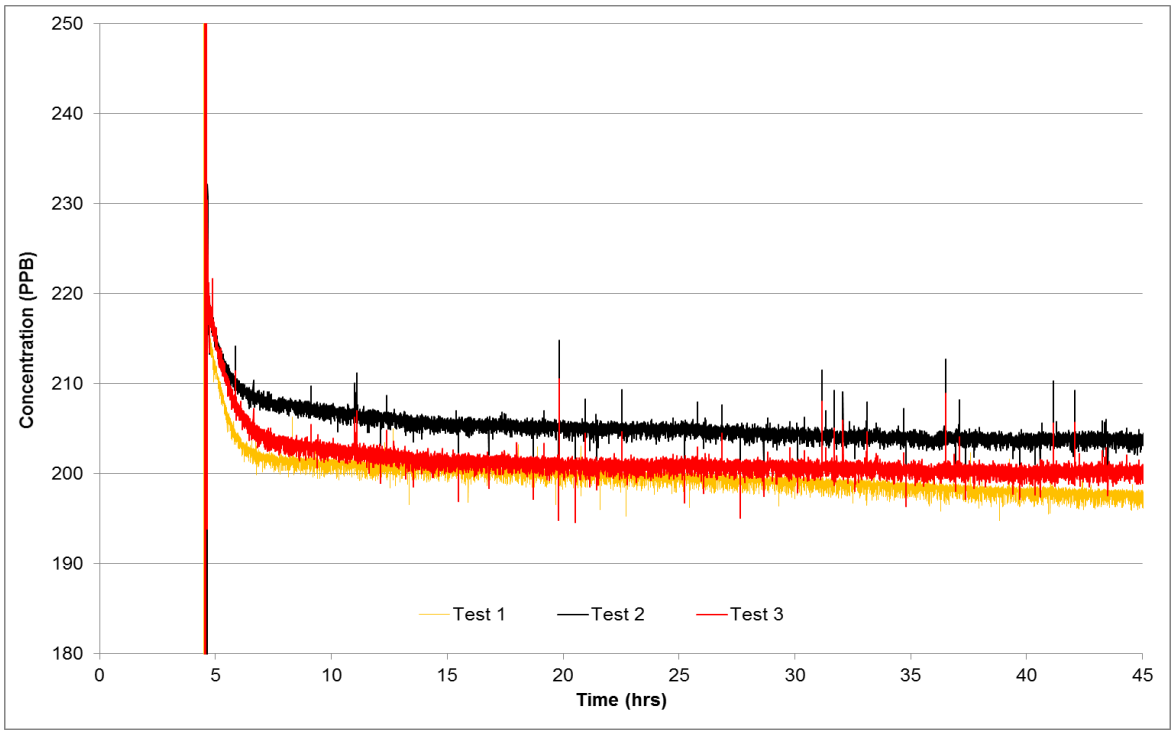


Figure 2.21a: Trace test outputs after temperature compensation in glass bead test (flat bed; 5 mm sediment particle size)

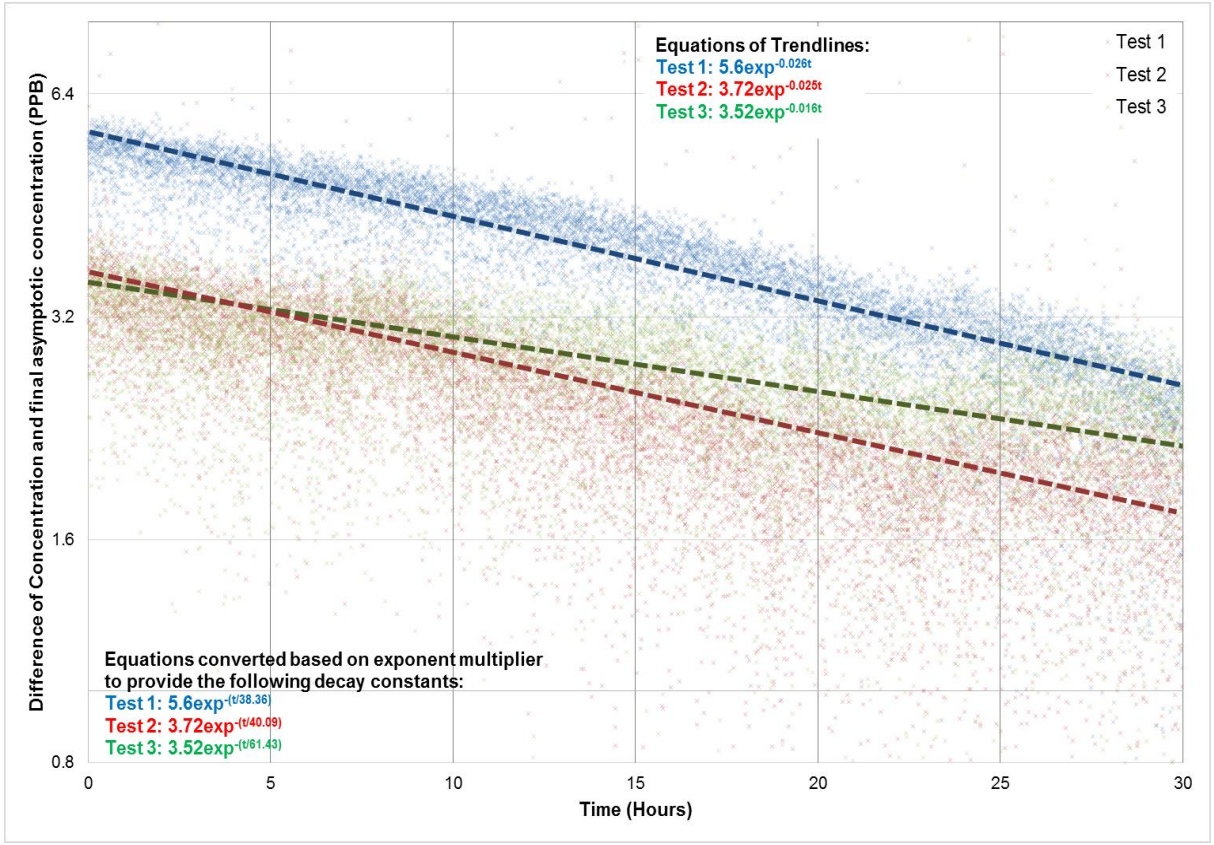


Figure 2.21b: Trace test outputs after temperature compensation in glass bead test (flatbed; 5 mm sediment) showing difference of concentration and final asymptotic concentration in log scale for 30 hours testing

2.4.2 Rhodamine Concentration Decay

Tests conducted with sediment material in place illustrated a continual decay of tracer concentration, which exceeded the expected end concentration levels (Figure 2.22). It was found that rhodamine did diffuse into clean water from gravel used in tracer tests (Figure 2.23), with concentrations approaching a plateau after 60 hours. The amount of rhodamine that was recovered was approximately 37 ppb, which was similar to the amount that had disappeared in the original trace (Figure 2.22). As Rhodamine has exceptionally low sorptive properties (Smart and Laidlaw, 1977) it was assumed that the Rhodamine had entered and collected in the pore spaces of the sediment, removing it from the water column over time and causing the gradual, yet continual, reduction in concentration seen in the tests. Figure 2.24a demonstrates that mass balance was achieved in the flume system with a synthetic bed material in place, irrespective of whether a single or multiple repeat discrete injections of tracer were used. Moreover, Figure 2.24b suggests that all three doses acted in a comparable manner.

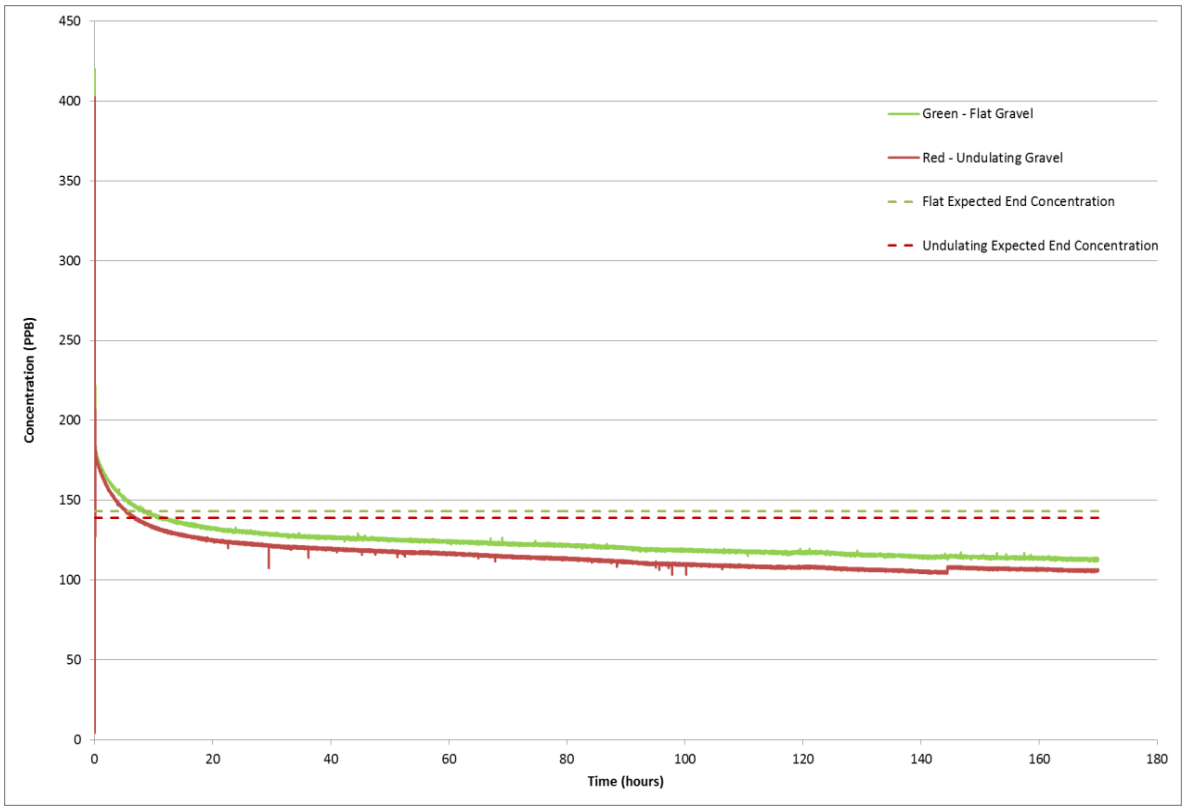


Figure 2.22: Trace test conducted on flat and undulating gravel beds at a discharge of 12 l/min

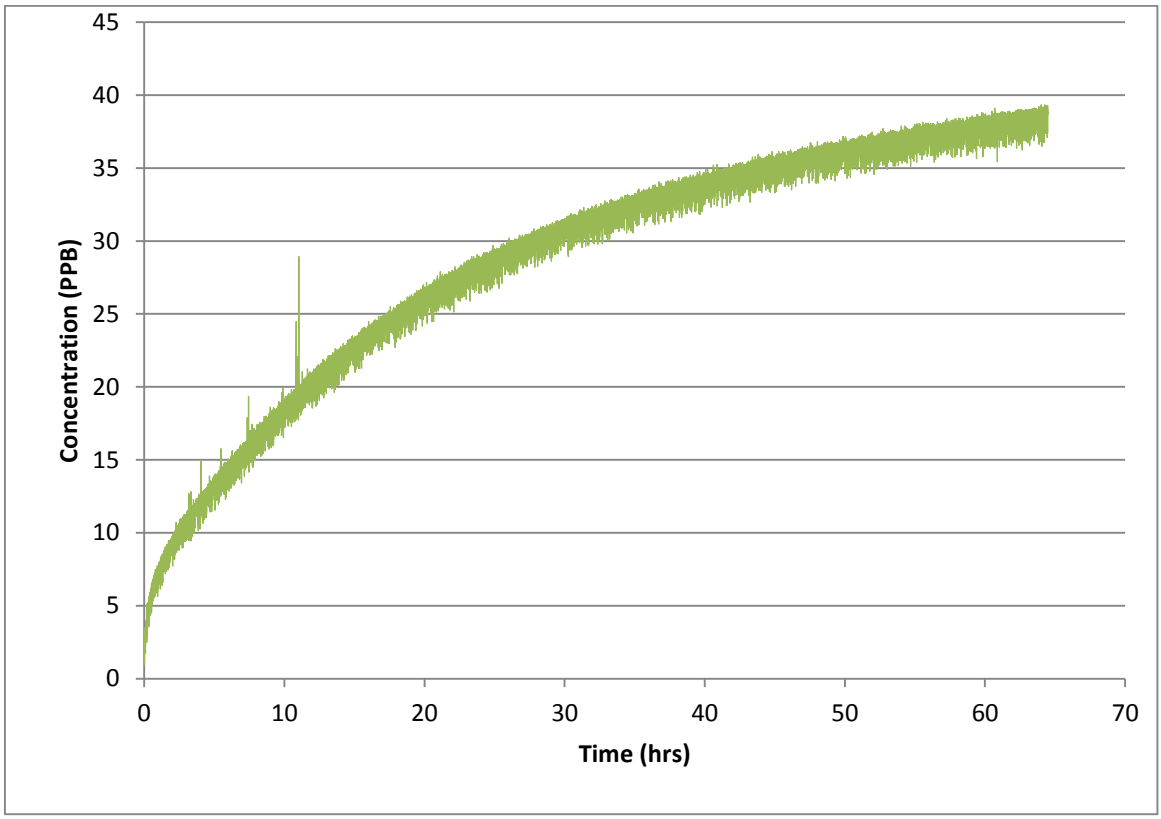


Figure 2.23: Reverse Sorption test trace; conducted at 12 l/min

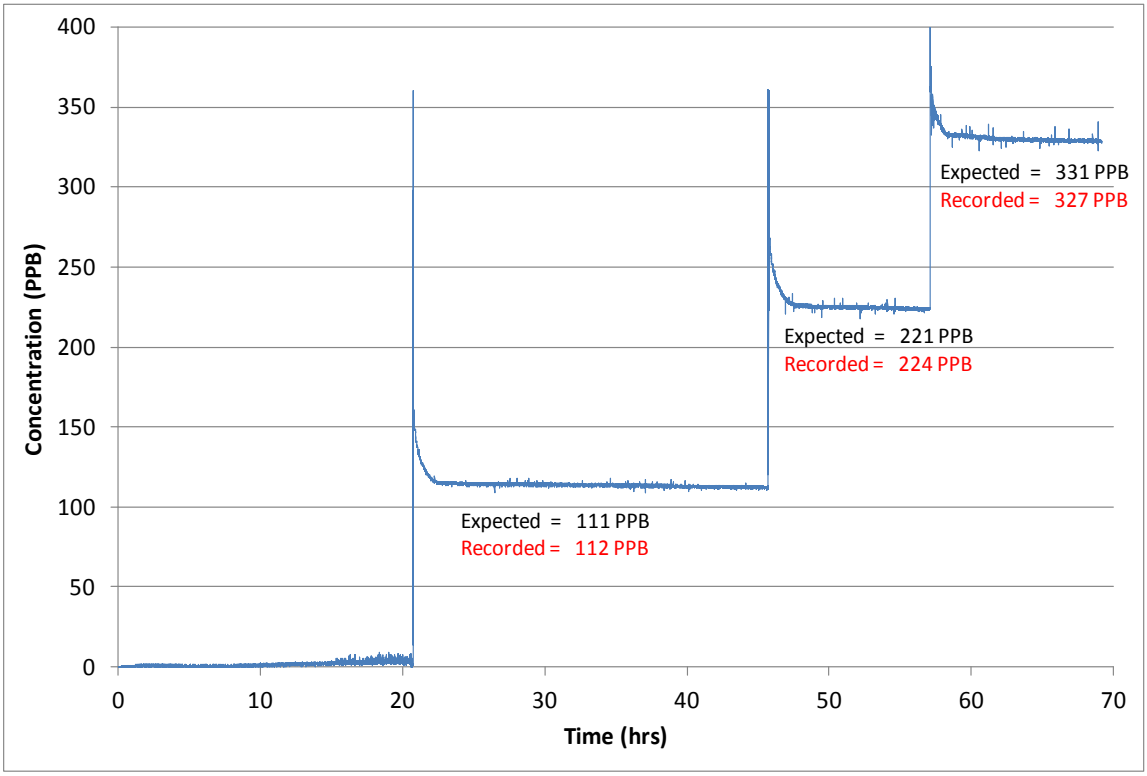


Figure 2.24a: Repeat discrete injection trace conducted on flat glass bead bed

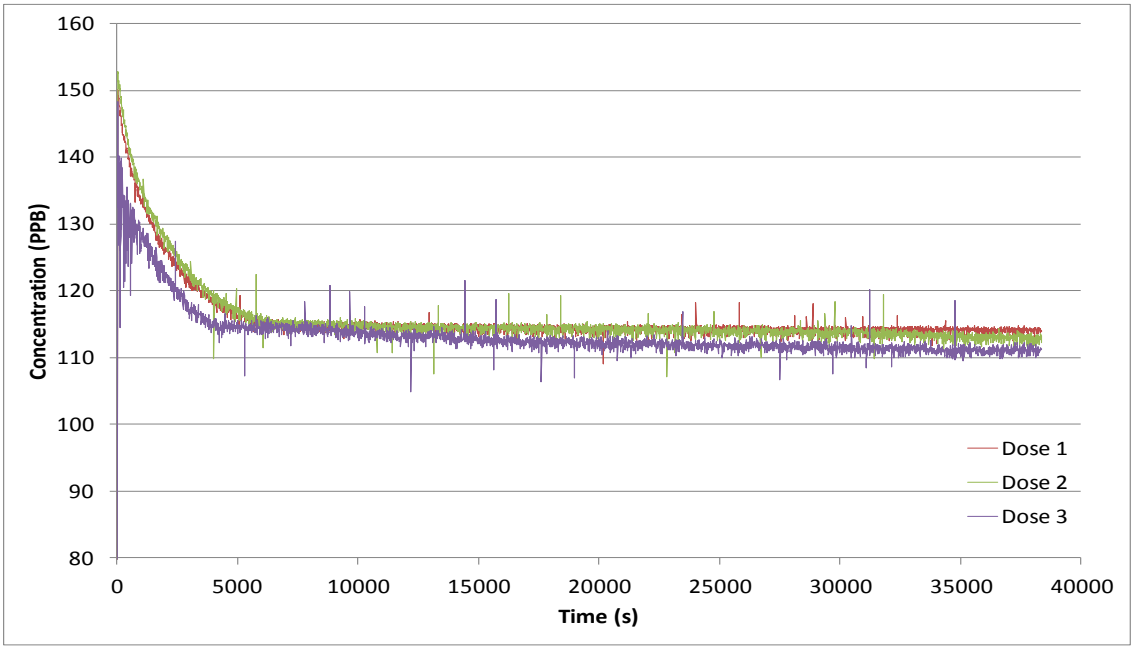


Figure 2.24b: Repeat discrete injection traces conducted on flat glass bead bed manipulated for gradient comparison

2.4.3 Consecutive Dosing

To establish whether time point injection affected mixing rates, trace tests without sediment present were conducted. Figure 2.25 and Table 2.2 shows that the tracer injections of Rhodamine into the water column reached the expected cumulative end concentration level with each continual tracer injection. Figure 2.26 shows the mixing response of the tracer in the water column with each repeat discrete injection when the trace data was manipulated to produce a visual comparison of each dosage addition-mixing phase. Figure 2.26 suggests that the system is capable of maintaining identical mixing rates irrespective of time of injection, shown by complete mixing within the water column occurring within 0.1 hours.

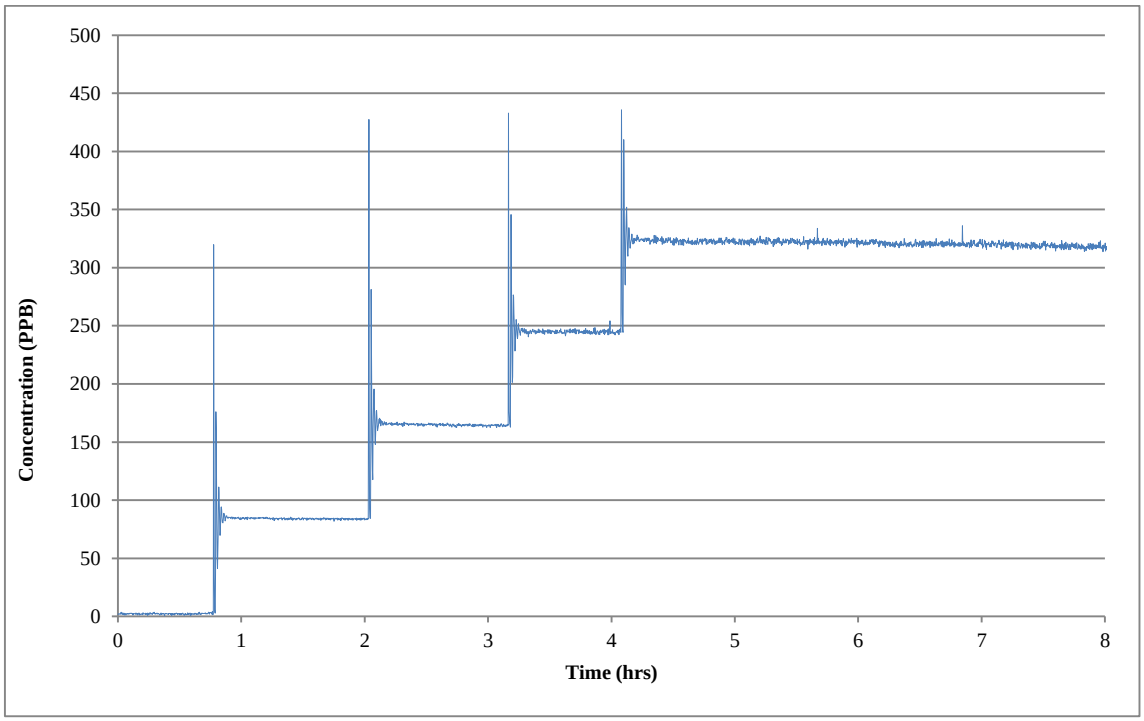


Figure 2.25: Temperature Compensated Raised Glass Bed Trace - 4cm Flow Depth

Table 2.2: Expected and Recorded End Concentrations at each Consecutive Dose

Dose	Expected End Concentration (PPB)	Recorded End Concentration (PPB)
1	80	83
2	160	164
3	240	243
4	320	327

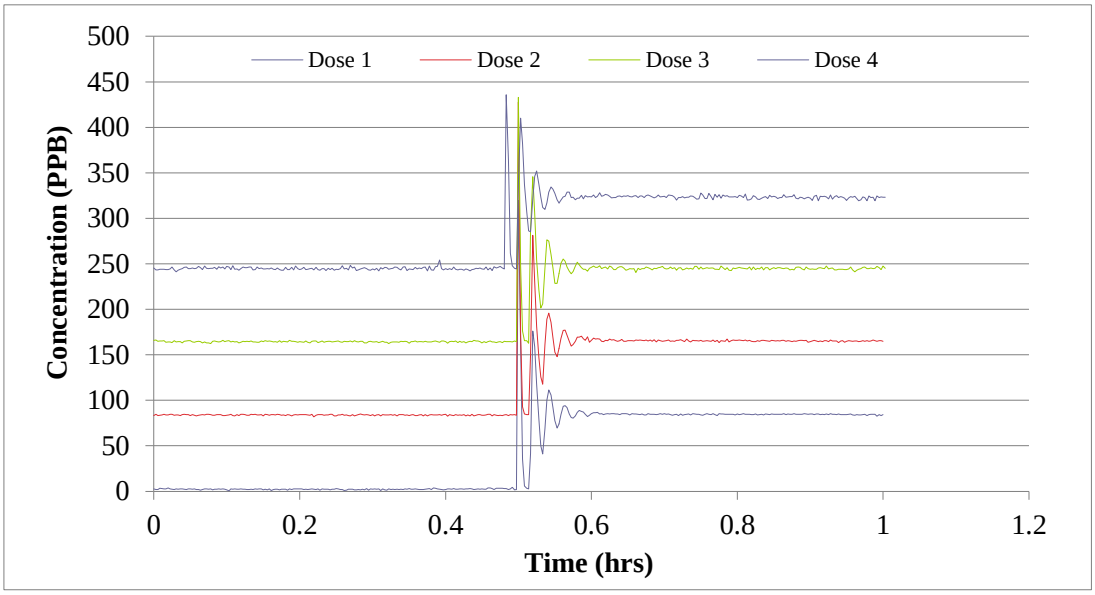


Figure 2.26: Visual comparison of consecutive doses added to raised fixed glass bed flume test

2.4.4 Sinusoidal Oscillations

Figure 2.27 shows that the sinusoidal oscillations disappeared when the black, red and yellow Cyclops heads were removed from the re-circulating system into separate water columns in disconnected flume channels, suggesting a connection existed between multiple instruments in a single water column and the oscillations. As each instrument was placed into an independent flume, the sinusoidal oscillations recorded by that instrument ceased.

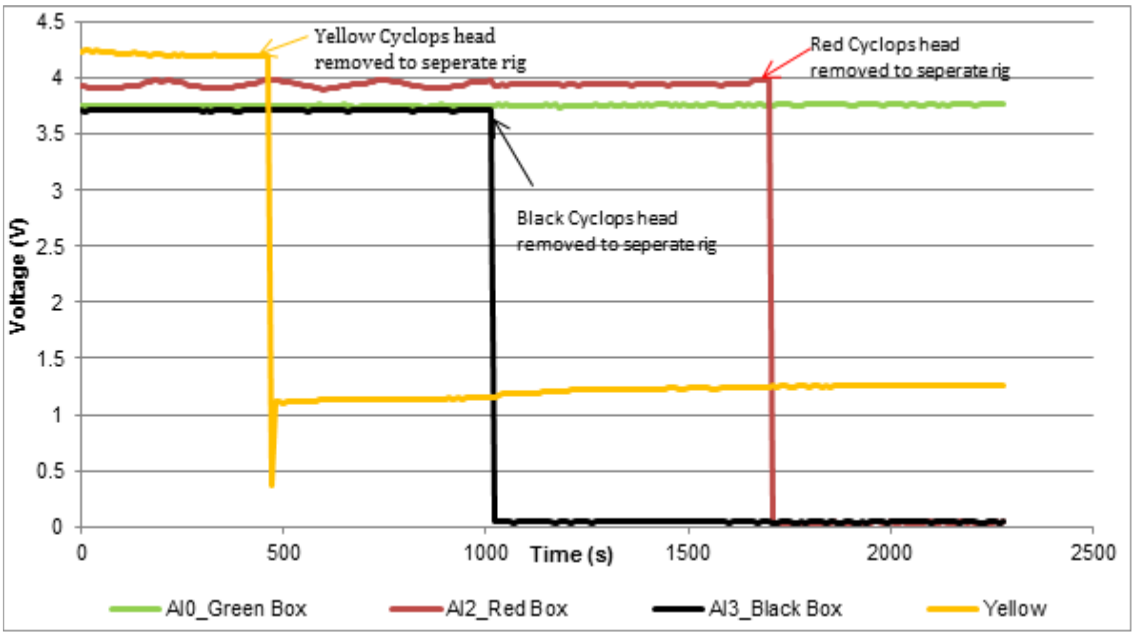


Figure 2.27: Removal of Cyclops fluorometers from water column of flume 8 to independent flume channels to assess impact on oscillation

2.5 Discussion

In order to complete the initial objectives of this study, provisional experimentation was required to ensure the reliability, functionality and reproducibility of the experimental systems prior to long term testing which would permit identification of biofilm development on hyporheic exchange. Initial tests focused on establishing the functionality of Rhodamine WT as a suitable chemical tracer for experimentation in the system. As Rhodamine WT is impacted by temperature at a known rate, it was possible to apply an algorithm to the data to account for temperature fluctuations (Smart and Laidlaw, 1977). The equation used in Smart and Laidlaw (1977) was reworked and applied to the recorded data to account for change in temperature from the starting run temperature over the course of the test which was identified as impacting the recorded fluorescence levels over time. This application resulted in plateaus being seen in the trace tests, indicative of mass balance in the system (within 5% of expected end concentration) with the absence of a sediment bed (Figure 2.21). As mass balance was achieved and found to be reproducible in the system without a sediment bed, testing was then able to progress to trace test analysis with bed material in the systems, acting as a porous bed.

However, employment of natural sediments in the experimental system resulted in tracer decay during testing (Figure 2.22). This supported a shift to experimental testing using synthetic glass beads as an artificial substrate. The utilisation of this artificial sediment enabled the systems to achieve a state of mass balance (Figure 2.24), thereby permitting progression to more advanced testing as the system had been established as stable. The employment of this substrate could be viewed as experimentally viable due to its recent employment in numerous fluvial projects to remove heterogeneity of substrate type from experimental studies (Chandler, 2013; Richardson and Parr, 1988; Nagaoka and Ohgaki 1990 and Lai et al., 1994). This resulted in the requirement of subsequent tests to isolate the cause of reduction in fluorescence levels.

With a continual temporal decrease in fluorescence levels surpassing the expected end concentrations further tests were needed. This sorption accounted for the majority of the decay seen in the gravel and sand traces. As such, to assess accurate hyporheic exchange gradients with a sediment bed it was necessary to utilise impermeable sediment that would not replicate the same issues seen with porous sediments. Research into other flume studies using Rhodamine as a tracer resulted in the decision to use glass beads as an alternate synthetic substrate (see O'Connor and Harvey, 2008). The employment of glass beads minimised sorption as a factor, as the synthetic glass beads were smooth and impermeable, and added uniformity to the bed sediment, removing variance in the bed material. Figure 2.24b indicates that mass balance was obtainable in the system over time and that the gradients produced from the trace are comparable when the substrate is not impacting the exchange or the tracer.

Moreover, Figures 2.25 and 2.26 indicated that the dosing methodology utilised in experimentation was reproducible and repeatable. This means that any change in fluorescence levels observed in the test systems after injection are attributable to the exchange dynamics as opposed to anomalies in the dosing method. Additionally, this affirms the fact that the system is suitable for repetitive dosing over time as the duration and fluctuation does not differ, making it experimentally viable.

Preliminary experimentation indicated that the oscillation interference was attributable to light feedback recorded on the LEDs incorporated in the Cyclops', directly related to interference fringe patterns from non-identical LED flash rates that made the beams of light within the channel out of phase with one another. This was problematic as flash timing, when superimposed, combines crests if they meet in the same phase (waves increasing or decreasing simultaneously). In the tests conducted where oscillations had been seen there was constructive interference, as the crest of a wave met the crest of another wave at the same point, resulting in the magnitude of the displacement being the sum of the individual magnitudes combined (Figure 2.28).

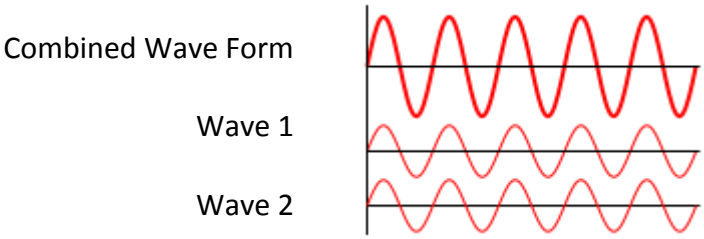


Figure 2.28: Diagram illustrating the creation of constructive interference from waves

The reflective properties of the system may have aggravated this matter by reflecting light around the system, causing it to be logged on other instruments. As such, all subsequent experimentation isolated the instruments in separate systems, meaning that no interference could be logged by the fluorometers. The isolation of the Cyclops' meant that experimentation could be conducted with confidence in the experimental set up. However, it did mean that the experimental methodology could only record fluorescence reduction from the water column and not monitor the increase in the sediment bed, inhibiting the level of potential monitoring.

The provisional experimentation has resulted in an experimental methodology that is reproducible, repeatable and capable of monitoring hyporheic exchange in the specially designed re-circulating flume systems. The methodology ensures that the only parameter recorded during experimentation is exchange and that all flume channels respond in an identical manner. The decision to employ uniformly sized glass beads as synthetic substrates in testing removed any inaccuracies that may have arisen as a result of sediment porosity and permeability. Additionally, the use of two different sediment particle sizes creating opportunity for comparison of microbial community development on different bed materials that have previously been studied in alternate flume studies investigating exchange. Moreover, the modification of the flow parameters for the final experimental design generated experimental flow conditions that mirrored those of lowland UK river systems in terms of turbulence and velocity.

Chapter 3

The interactions between bed morphology, sediment particle size, biofilm development and hyporheic exchange in re-circulating flume systems

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3.1 Introduction

Hyporheic exchange is the process by which solutes are transferred between the overlying water column and the interstitial fluid within fixed bed sediments. This form of stream-subsurface exchange has received increased investigation due to intensified awareness of its control of contaminant and nutrient fluxes (Marion *et al.*, 2002). This is a result of its impact on the residence time of chemicals in the environment and their distribution. The sediment-water interface represents an assemblage of biological communities, mineral particles, organic material and void spaces which form a boundary for fluxes of solutes, water and particulates (O'Connor and Harvey, 2008). Exchange is predominately driven by three fundamental processes; pressure gradients, diffusion (molecular and turbulence) and concentration gradients (Chow, 1959).

In recent years, quantification of hyporheic exchange has become a key focus of research, which has resulted in a detailed review of analytical methodology (Battin and Sengschmitt, 1999; Bottacin-Busolin *et al.,* 2009; O'Connor and Harvey, 2008; Marion *et al.,* 2002). However, this work has predominantly focused on the role of physical parameters (bed-form, discharge, flow depths and bed depths) in determining exchange without taking into account biological considerations such as microbial characteristics and their spatial distribution.

Recent research into hyporheic exchange has been undertaken in controlled *ex-situ* systems to eliminate uncontrollable variables that predominate rivers and inhibit controlled experimentation (Friedrich *et al.*, 2007). Laboratory studies permit simulation of natural environments in a simplified manner using water

and sediment in quantities small enough to control environments and elucidate process rates and characteristics (Friedrich *et al.*, 2007; Williams, 1970).

3.1.1 Hyporheic Exchange

The hyporheic zone is most commonly identified as the interstitial porous sediment beneath and adjacent to a column of water (White *et al.*, 1995). Figure 3.1 illustrates hyporheic exchange, where the overlying water column transfers solutes into the interstitial solution surrounding porous sediments due to concentration gradients and flow variations (Cardenas *et al.*, 2004). The exchange that occurs within this zone play fundamental roles in the hydrology, material and nutrient cycling and energy flows in lotic systems, with the majority of biogeochemical processes occurring within it (Boulton et al., 1998; Pusch, *et al.,* 2002; Nogaro *et al.,* 2010). Consideration of hyporheic exchange is therefore crucial when estimating contaminant fluxes in lotic systems; particularly as the bed material and bed form have the potential to control pollutant distribution patterns (Rutherford, 1994; Wondzell, 2006).

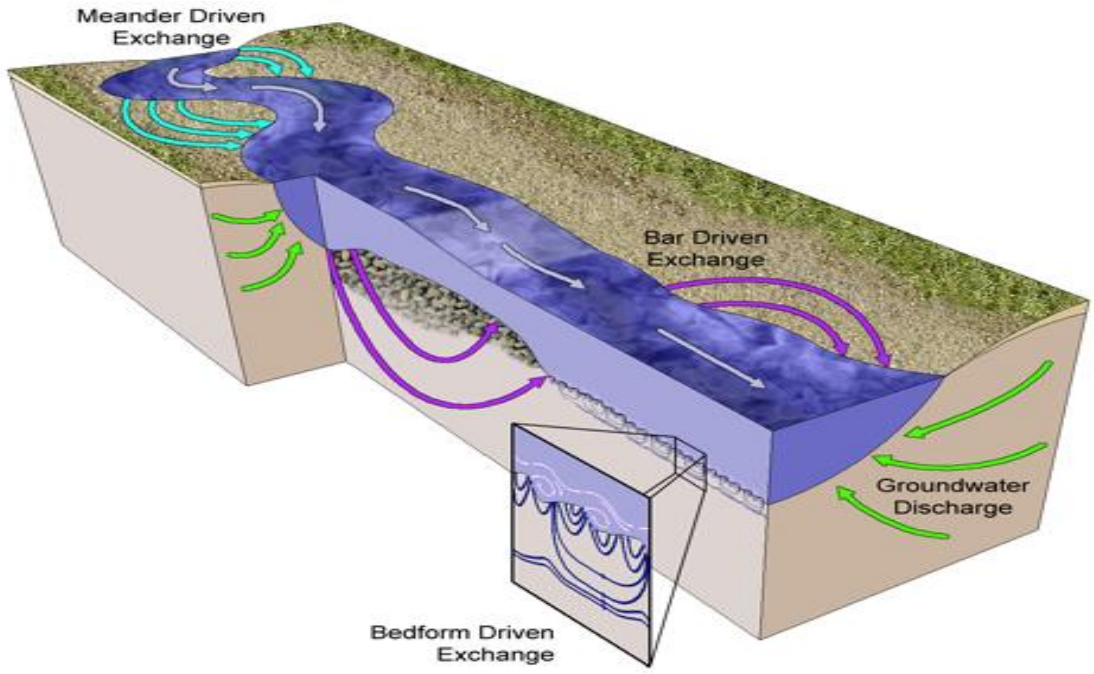


Figure 3.1: Hyporheic exchange is the interchange of surface-water with ground water in the hyporheic zone, associated with concentrations gradients, bed-form variations and other natural processes
(Source: http://susa.stonedahl.com/research.html)

3.1.1.1 Solute Transport

Chemical, biological and physical processes influence solute transport in rivers, and its rate is dependent upon both the environmental parameters and the solute's properties (Ng and Yip, 2001). Following point source pollution events, the point downstream that the pollutant reaches is affected by solute transport mechanisms which impact the rate of hyporheic exchange of soluble materials from the overlying water column to the interstitial fluid in the sediment bed, across the sediment-water interface (Dutton, 2004; O'Connor and Harvey, 2008). Moreover, the geomorphological, chemical and biological processes that occur within the river channel will impact the degradation of the pollutant. As such, chemical degradation in lotic systems is difficult to predict due to the variance in environmental parameters that exist in the natural world.

3.1.1.2 Molecular Diffusion

Molecular diffusion, in its simplest form, is the thermal motion of all particles (liquid or gas) at temperatures above absolute zero. The speed of this movement is a function of fluid viscosity, the size/mass of the particles and temperature (Rutherford, 1994). This form of diffusion predominately explains the net flux of molecules from a region of higher concentration to one of lower concentration. Molecular diffusion has often been identified as the driving transport method when studying biogeochemical gradients in sediments (O'Connor and Harvey, 2008), making its quantification paramount for predicting exchange.

3.1.1.3 Effective Diffusion

Effective diffusion is a measure of all physical transport mechanisms within the fluvial system. There are many different parameters and processes that can impact hyporheic exchange. In recognition of this, and also to aid in the comparison of hyporheic exchange coefficients driven by different processes, this collaborative process is referred to as effective diffusion, which is often used to create coefficient for overall exchange (O'Connor and Harvey, 2008).

3.1.1.4 Predicting Scaling Relationships

The majority of studies investigating hyporheic exchange have resorted to using artificial watercourses in the laboratory to enhance understanding of fluvial processes by assessing the role of individual factors affecting river systems independently (Williams, 1970; Packman and Bencala, 2000).

Data obtained from such studies have been collated and used to examine the effects of varying fluid-flow and sediment conditions on transport in permeable sediments, thereby generating effective diffusion scaling relationships to quantify exchange (O'Connor and Harvey, 2008). Irrespective of the differences in the data collection methodology, the tests conducted showed it was possible to quantify and model the effective diffusion coefficient for given differing river systems, thereby deriving a rate of hyporheic exchange. O'Connor and Harvey's (2008) hyporheic exchange coefficient was effective with differing test methodologies, as the majority of the studies investigated sediment and flow parameters but merely recorded the outputs in a different manner, measuring either tracer concentration reduction from the water column (Lai et al., 1994; Elliott and Brooks, 1997b; Marion et al., 2002; Packman et al., 2004; Packman et al., 2000; Packman and MacKay, 2003; Ren and Packman, 2004; Rehg et al., 2005; Tonina and Buffington, 2007) or increase within the interstitial fluid (Elliott and Brooks, 1997b; Nagaoka and Ohgaki, 1990; Shimizu et al., 1990). Table 3.1 shows the experimental parameters and experimental studies utilized by O'Connor and Harvey (2008). O'Connor and Harvey (2008) specified scaling constant and exponents by plotting three dimensionless numbers against the dimensionless diffusion coefficient, which demonstrated a strong correlation between the Péclet permeability number, which is a measure of permeability, and the shear Reynolds number, which is a dimensionless measure of shear rate. Figure 3.2 illustrates this scaling relationship (slope of 0.55) and its accuracy (O'Connor and Harvey, 2008).

It has been shown that environmental variables have the capacity to impact these relationships, and affect the rate of exchange (Packman et al., 1997;

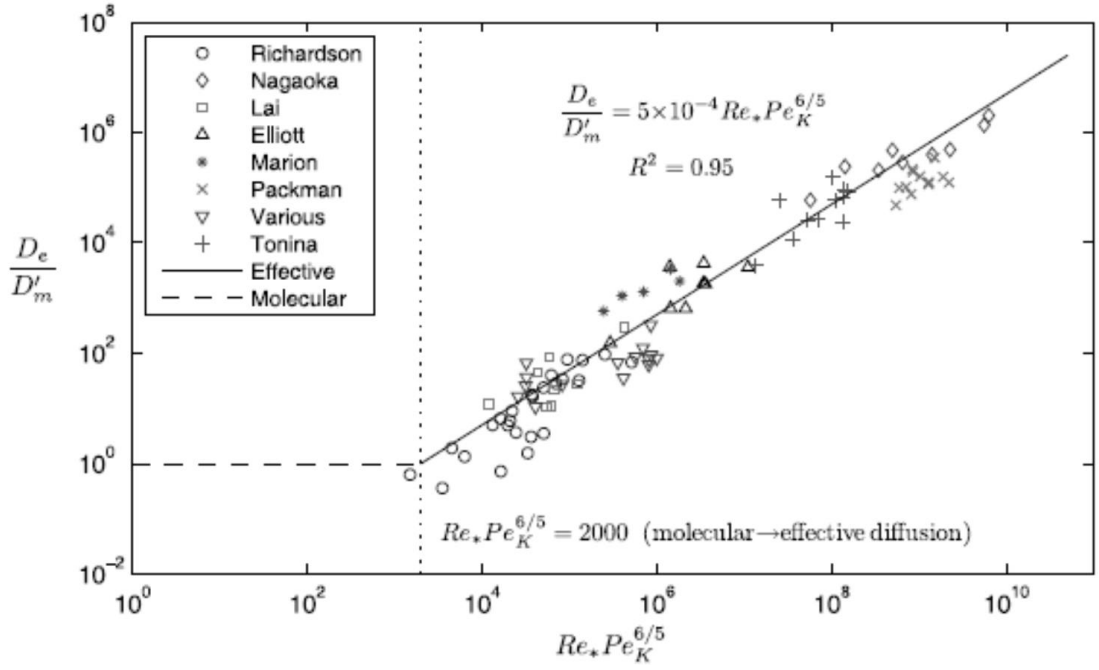


Figure 3.2: O'Connor and Harvey's (2008) Predicted scalar relationship (Re_* = Shear Reynolds Number; Pe_K = Permeability Péclet Number)

Nogaro *et al.,* 2010). Although studies have been conducted to identify and quantify the effect of variability in sediment, flow and bed-form on hyporheic exchange, little to no investigative work has been conducted on the impact of ecological processes that modify the organization and function of the hyporheic zone (Hendricks, 1996; Fischer et al., 2005; Nogaro *et al.,* 2010).

Table 3.1: Experimental parameters utilized by O'Connor and Harvey (2008)

Study	d_g (mm)	K (10^{-6} cm^2)	θ	Δ (cm)	λ (cm)	u_* (cm/s)	U (cm/s)	H (cm)
Richardson[a]	0.1-3.0	0.17-71	0.36-0.40	0	0	0.3-1.3	3.7-22.9	0.6-1.9
Nagaoka[b]	19.0-40.8	500-2300	0.24	0	0	1.1-4.3	8.9-42.8	3.2-7.0
Lai[c]	0.5-3.2	2.3-19	0.36-0.38	0	0	0.2-0.6	7.4-15.4	0.5-2.0
Elliott[d]	0.1-0.5	0.08-1.1	0.30-0.33	1.1-2.5	9-30	1.3-2.4	8.6-13.2	3.1-6.5
Marion[e]	0.85	5.0	0.38	0-3.5	0-120	1.7-1.8	22.0-28.0	10.9-12.3
Packman[f]	4.8	150	0.38	0-3.7	0-32	1.1-3.2	9.0-36.1	11.3-20.5
Various[g]	0.5	0.68-1.8	0.29-0.38	0.8-1.9	15-70	0.5-1.7	12.0-23.7	7.1-12.7
Tonina[h]	9.8-10.8	51	0.34	3.6-12.0	515-560	3.8-5.5	28.2-46.0	3.9-10.4

Where d_g is geometric mean particle size; K is permeability; θ is porosity; Δ is bed-form height; λ is wavelength; u_* is shear stress velocity; U is average velocity; H is flow depth

(***a.*** Richardson and Parr, 1988; ***b.*** Nagaoka and Ohgaki, 1990; ***c.*** Lai et al., 1994; ***d.*** Elliott and Brooks, 1997b, ***e.*** Marion et al., 2002; ***f.*** Packman et al., 2004; ***g*** Packman et al., 2000; ***g.*** Packman and MacKay, 2003; ***g.*** Ren and Packman, 2004; ***g.*** Rehg et al., 2005 and ***h.*** Tonina and Buffington, 2007)

3.1.1.5 Hyporheic Exchange Tests

Previous studies have shown how different environmental parameters possess the capacity to impact river systems, such as persistence of chemicals, channel morphology and physical disturbance (Lake, 2000; Vörösmarty *et al.*, 2010). These factors have the capacity to impact the extent to which pollutants carried in water come into contact with degrader communities inhabiting the sediment boundary, which are influenced by a range of factors, including the solubility of the chemical and mixing characteristics (Nogaro et al., 2010). The mixing characteristics may be governed by flow rate, sediment composition and channel morphology, particularly bed-form (Marion *et al.*, 2002; Bottacin-Busolin *et al.*, 2009).

It has been established that undulating bed-forms influence flow resistance and exchange, causing flow separation and associated energy dissipation (Vanoni and Hwang, 1967; Wijbenga, 1990; Ogink, 1988; Julien et al., 2002). Research has demonstrated that the larger the amplitude of the bed-form, the greater its impact on exchange (Elliott and Brooks, 1997; Marion et al., 2002; Packman et al., 2004). However, the channel of flow through undulating bed-forms has never been examined in detail. This lack of exploration means that there is limited knowledge of the connectivity between bed-forms of different wavelengths in river systems, the potential variability of which is shown in Figure 3.3. This could mean that nutrients within the flow have the potential to penetrate to deeper depths in bed-forms with smaller wavelengths, generating opportunity for supporting greater microbial populations at depth.

Some studies, such as the work of Nogaro et al., 2010 have suggested that the influence of sediment clogging by microbial processes is complex, requiring further experimental investigation to accurately quantify and potentially model the impact of microbial development on exchange (Nogaro et al., 2010).

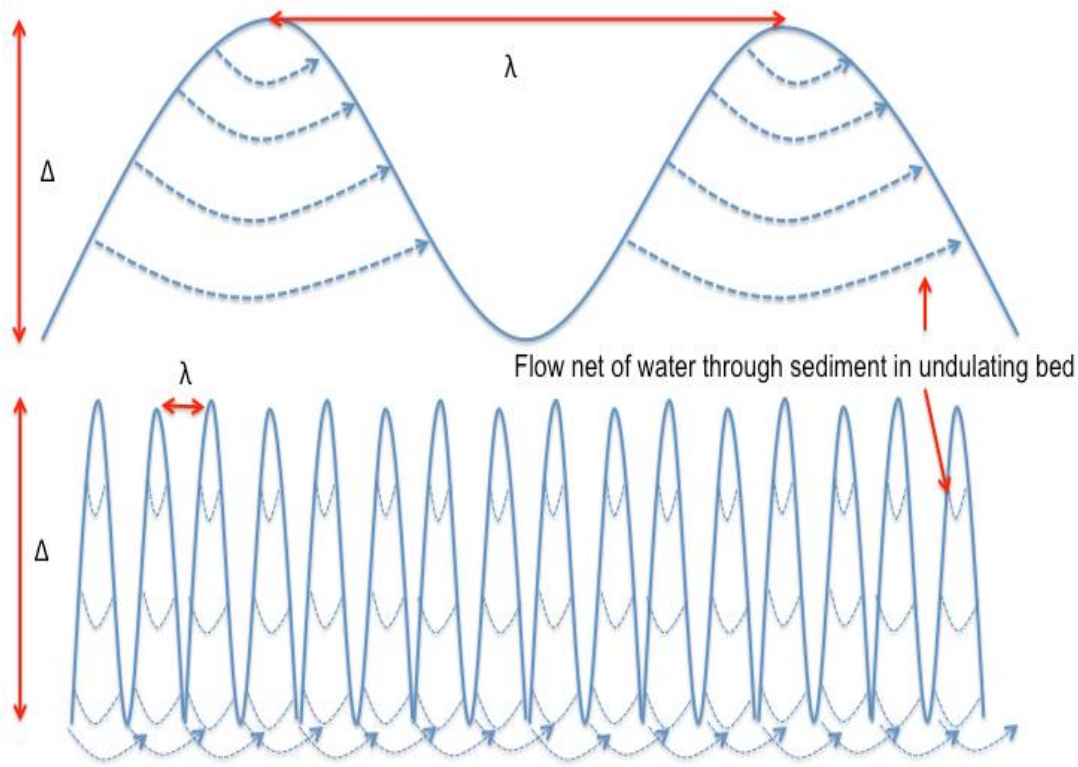


Figure 3.3: Illustration of the greater potential depth of flow through an undulating bed-form with a smaller wavelength due to increased connectivity between the dunes

3.1.2 Impact of Biofilms on Exchange

Microorganisms inhabit every possible environment that can support life, impacting human practices at all scales (Madigan and Martinko, 2006). In natural systems microorganisms are closely associated with surfaces and interfaces as multicellular aggregates collectively held by the extracellular polysaccharides they secrete (Wimpenny *et al.*, 2000). This collection of microorganisms within an extracellular polymeric substance (EPS) is referred to as a biofilm (Flemming *et al.*, 2007). Biofilms are derived sessile communities characterised by the cells that are attached to a substratum or interface. Experimental studies have indicated that biofilm bacteria predominate in all ecosystems, resulting in these sessile organisms impacting industrial, medical and environmental processes (Costerton *et al.*, 1995).

Furthermore, all previous work on identification of bed-form impacts on exchange in lotic systems has identified that the amplitude of the bed-form affects exchange irrespective of the wavelength of the dune (Elliott and Brooks, 1997; Marion et al., 2002; Dutton, 2004; Packman et al., 2004; Tonina and Buffington, 2007; O'Connor and Harvey, 2008). However, no work has been conducted to determine how wavelength affects biofilm formation through the bed profile and whether this impacts hyporheic exchange.

To assess the environmental risk of specific chemicals, information allowing the estimation of their likely concentrations in the environment is required (OECD Guideline for testing of chemicals, 2005). Chemical biodegradation is one of the most important aspects determining environmental behaviour and is required for chemical classification purposes (Rudén and Hansson, 2010). However, the interactions between bed-form, microbial biofilm communities, chemical biodegradation and other environmental variables at the sediment-water interface are not considered in standardised OECD testing protocols or modelling for chemical risk assessment (Ericson, 2007). Water–sediment exchanges have been found to modify water flow patterns (Nogaro *et al.,* 2010) and influence the growth of microbial communities in sediments (Brunke & Gonser, 1997; Battin, 2000) which can clog interstitial pore spaces in sediment

beds, thereby influencing microbial growth and activities in the sediment layers (Nogaro *et al.,* 2010). *In-situ* studies have identified that sediment deposition induces severe clogging (Nogaro *et al.,* 2010). However it has also been noted that microbial development differs between experimental sites and can result in different levels of interstitial sediment clogging at each location, potentially impacting hyporheic exchange at different rates (Nogaro *et al.,* 2010). This is shown in Figure 3.4.

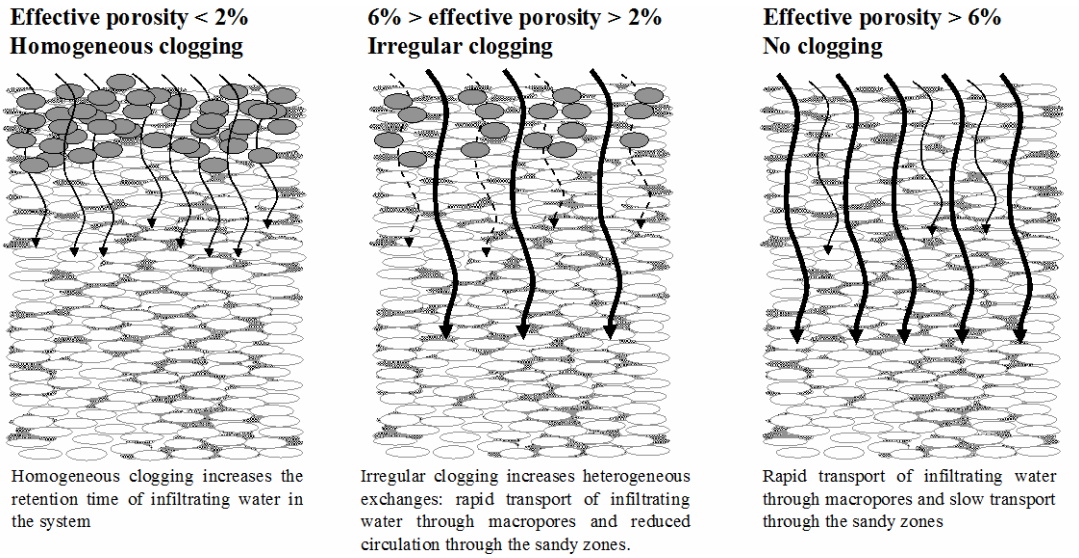


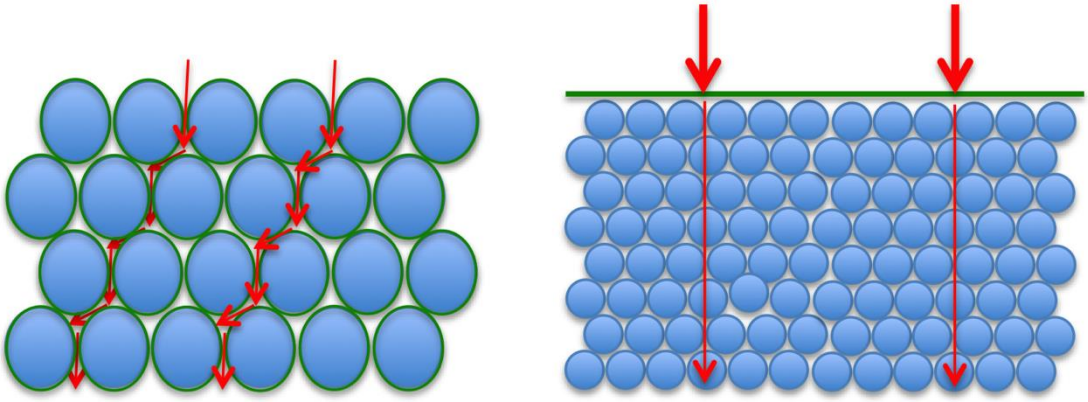
Figure 3.4: Clogging effects of microbial communities depending on the effective porosity of hyporheic sediments; taken from Nogaro et al., 2010

3.1.2.1 Sediment Clogging

Influxes of nutrients, chemicals and pollutants are dispersed to biofilm organisms embedded in EPS via diffusion. However, hydrodynamic processes can exert control over the functioning and location of biofilms as flow forces alter the persistence of the EPS and cellular aggregates (Elliott and Brooks, 1997). Conversely, evidence suggests biofilms affect the hydrodynamic processes in porous bed sediments (Battin and Sengschmitt, 1999), with biofilm accumulation reducing the hydraulic conductivity of saturated sediment, clogging porous sediments and altering hyporheic exchange rates. Although this clogging of porous media has the capacity to stabilize cohesive sediment and reduce fluvial erosion, it is believed that the presence of biofilms may impact the rate of hyporheic exchange by impeding solute transfer (Bottacin-Busolin et al., 2009; Nogaro et al., 2010).

Investigative studies conducted *in-situ* have suggested that sediment clogging, by particulate sediments and biofilm communities, vary according to sediment particle size, nutrient source, geography, climate and site variability (Nogaro et al., 2010). These studies found that biofilm development varied dependent upon the sediment composition, supporting the need for further *ex-situ* lab based studies to accurately quantify the extent of biofilm community development on different sediment surfaces and particle sizes.

It can be argued that fine sediments possess the potential to inhibit infiltration of nutrients to depths comparable to those in the larger sediments due to the longer expected hyporheic mixing times, based on previous experimental works (see O'Connor and Harvey, 2008). This is because the nutrients are not able to penetrate as deep into the sediment bed as they would be able to in larger sediments, restricting the infiltration capabilities of the nutrients. It was believed that the finer the sediment the smaller the infiltration depth of biofilm development and, therefore, the thicker the layer of surface biofilm development. This is attributable to differences in aquatic microbial community development over finer scales than those usually identified in microbial research (Smart et al., 2008). This is illustrated in Figure 3.5.



- Biofilm develops around the surface of each large independent particle (5 mm) which may affect hyporheic exchange
- As the biofilm development does not clog the interstitial porous spaces in the sediment it may be unlikely to significantly decrease the rate of exchange

- Smaller sediment particle size (0.5mm) permits the microbial community to develop as a 'mat' over the surface of the sediment
- This may have the potential to inhibit the rate of hyporheic exchange

Figure 3.5: Illustration of differences between theoretical potential biofilm developments on different sediment particle sizes

3.2 Objectives

This investigation aimed to explore the effect of bed-form characteristics on the spatial variability of microbial biofilm community development at the sediment-water surface, and its subsequent impact on hyporheic exchange. Effects of biofilm development on exchange were examined using the series of specially designed re-circulating flume systems described in Chapter 2, which replicated actual channel flow. The objectives of this Chapter were to:

1) Determine the effect of sediment particle size on the rate of exchange in an experimental flume

2) Elucidate the difference in rate of exchange between flat and undulating beds in the flume systems

3) Determine how sediment particle size affects biofilm development

4) Quantify the impact of biofilm on the rate of exchange as a result of clogging of interstitial pore spaces in flat bed systems

5) Identify whether the wavelength of dunes affects biofilm development and its subsequent impact on hyporheic exchange

3.3 METHODS

3.3.1 Determine effect of sediment particle size on the rate of exchange in an experimental flume

To assess the exchange of solutes into sediment from the water column, fluorescent dye trace experiments were conducted on different sediment particle size beds to quantify the impact on the rate of exchange. This experimental protocol was comparable to methodologies employed in other flume studies that have quantified bed-form driven exchange (Elliot and Brookes, 1997; Marion et al., 2002; Dutton, 2004).

3.3.1.1 Experimental Apparatus

Two sediment particle sizes were used to determine the effect of particle size on hyporheic exchange rates. Both sediments were uniform particle sized synthetic glass beads with mean particle sizes of 5 mm (representative of gravel) and 0.5 mm (representative of coarse sand) (VWR International glass beads). The 5 mm sediment size is representative of those found in gravel bed rivers, whereas the 0.5 mm size glass beads are representative of sandy/fine sediments.

Prior to experimentation, the flume was cleaned and degreased using ethanol and the weirs were set in place to establish uniform flow. The uniform particle size synthetic glass beads were washed prior to use to remove any organic matter or fine particulates that may have accumulated on them. The sediments were then poured into the bed section of the re-circulating rectangular glass flume channels (0.01 m thick glass; 2.36 m long, 0.1 m wide) to a mean bed height of 10 cm. This was in keeping with the experimental design developed in Chapter 2. Following this, templates were used to create flat bed profiles to ensure repeatability of test parameters. Examples of these bed profiles can be seen in Figure 3.6. These tests were completed in triplicate.

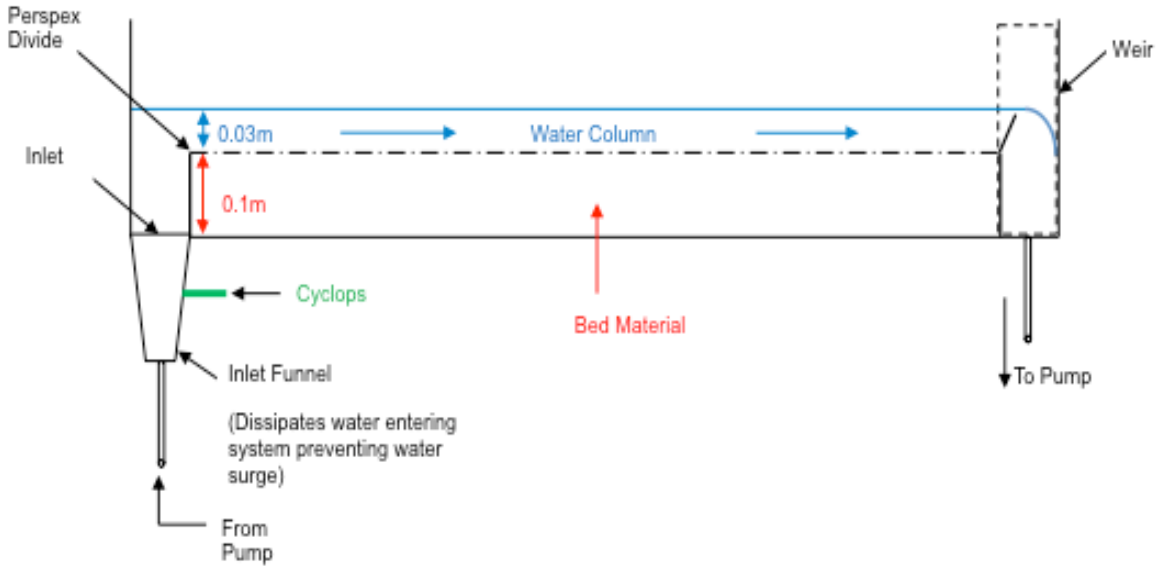
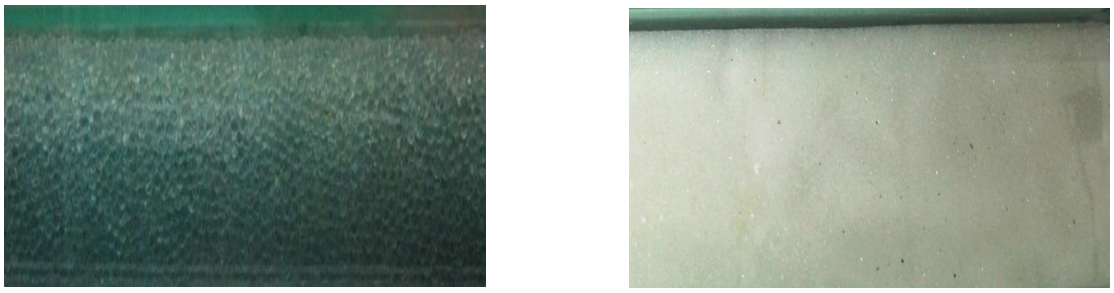


Figure 3.6a: Example flat bed profile employed in flume systems to quantify impact of particle size on rate of hyporheic exchange



5 mm Glass Beads 0.5 mm Glass Beads

Figure 3.6b: Photograph of sediment in the flume systems

3.3.1.2 Experimental Design

The bed section of flume system was filled to a bed height of 10 cm with appropriate sized glass beads to act as a synthetic substrate. Bed-form templates were then used to create each profile and sediment weight used in each system was logged to monitor any change in bed characteristics.

Turner Design Cyclops 7 Fluorometers were placed in the inlet section of the flume system to record levels of fluorescence in the water column during the flume test. Water for each test series, collected from the same location of the River Dene (Chapter 2) was filtered using a 38 μm mesh to remove any large particulate matter prior to testing in flume system. The flumes were then slowly filled with the water and uniform flow was established in each of the three flumes at a flow depth of 3 cm and a discharge of 12 l min^{-1} (replicated in each test); as described in Chapter 2. For this experimental series it took approximately 20 litres of water to establish uniform flow at a depth of 3 cm.

To assess the rate of exchange between the water column and the interstitial pore spaces in the sediment bed of different sized sediment particles, the fluorescent tracer Rhodamine WT (Smart and Laidlaw, 1977) was introduced into the centre of the channel at the weir. The Rhodamine solution was made up of concentrated Rhodamine (10^6 ppb) that had been diluted using water extracted from the flume system. The use of water from the system to create the Rhodamine tracer ensured that uniform flow was maintained, as there was negligible increase in water volume, in addition to ensuring the temperatures were comparable. The tracer was injected into the system using a funnel system that drip-fed it into the channel over the course of one complete re-circulation (48 ± 1 second). The amount of Rhodamine added was designed to give an increase in fluorescence to 30 ppb by the time equilibrium had been reached. As the tracer mixed in the water column and passed the measurement position in the inlet of the flume channel, a Turner Designs Cyclops 7 fluorometer recorded the change in fluorescence in the channel; as the system continued recirculating it was possible to monitor the rate of exchange of the

tracer into the sediment bed until complete mixing occurred and equilibrium was reached.

Within the controlled environment room, light was used to promote algal, and hence biofilm, development. Fluorescent daylight bulbs were covered with Lee DS 226 UV filter screens to prevent transmission of light wavelengths < 380 nm (Lee Filters, Andover, UK). The lights were on a timer to provide 16 hours light and 8 hours dark. Temporal differences in exchange due to sediment particle size were determined using O'Connor and Harvey's (2008) exchange coefficient analysis (Chapter 2).

Each sediment had its own bed profile and was allocated a bank of three flumes to enable testing in triplicate. Each triplicate set of tests was conducted on the same bank of flumes, as they required the same bed-slope.

3.3.2 Elucidate the difference in rate of exchange between flat and undulating beds in the flume systems

To assess the difference in penetration of solutes at the water-sediment interface as a result of variance in bed-form, fluorescent dye trace experiments were conducted on different bed-profiles, quantifying the impact of bed morphology on the rate of exchange in the experimental flume system. This experimental undertaking is comparable to methodologies employed in other flume studies (Elliot and Brookes, 1997; Marion et al., 2002; Dutton, 2004).

3.3.2.1 Experimental Apparatus

The experimental system utilised in this test was identical to that employed in section 3.2.1.1. Sediments of two different particle sizes (5 mm and 0.5 mm) were cleaned and used for experimentation. River water was taken from the same location of the River Dene used previously, and processed as described in section 3.2.1.1.

Wooden templates were created to prepare each bed profile. The templates permitted manipulation of the surface sediment to form the desired bed-form. The bed-forms and flow depths used in this experiment were as illustrated in Figure 3.7.

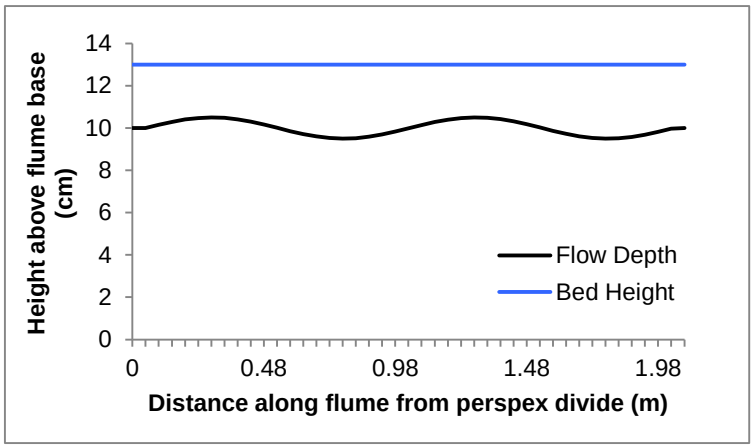
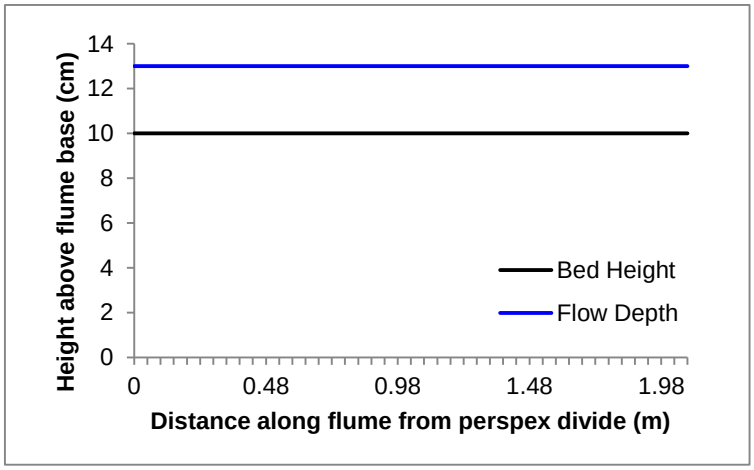


Figure 3.7a: The undulating bed-form employed in trace testing



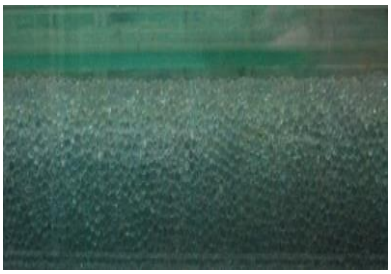


Figure 3.7b: The flat bed-form employed in trace testing

3.3.2.2 Experimental Design

The comparative rate of hyporheic exchange between flat and undulating bed-forms was recorded as described in Chapter 3, Section 3.2.1.2. Due to the variation in bed-form, it should be noted that the water volumes and flume elevation were modified to create uniform flow.

3.3.3 Determine how sediment particle size affects biofilm development

A series of experimental tests were devised to determine how sediment particle size impacts biofilm development and hyporheic exchange.

3.3.3.1 Water Collection Site

The water used for this test series was collected in the same manner as that described in Section 3.3.1.2. The water was collected from the study site described in Chapter 2, Section 2.2.1.4.

3.3.3.2 Experimental set up

To determine how sediment particle size affects biofilm development, a range of 5 different sediment particle sizes were used. To ensure that the sediments were homogenous in size, synthetic silica glass beads (VWR International) were used as a synthetic substrate in the experiment. Prior to experimentation, the beads and experimental units were autoclaved (121 ˚C, for 15 minutes, 1.1 atm) to ensure that no contaminants were present in the experimental system prior to addition of the inoculum.

Clear, sterile Schott glass beakers (500 mL) (Fisher Scientific, UK) were used as experimental vessels for this experiment. The beakers were filled with 50 g of homogenous particle sized glass beads, with river water then added as an inoculum (150 mL). The sides of the flasks were covered in foil to limit light penetration to the surface of the sediment, making it comparable to river sediments. The top of each beaker was then covered with cling film to inhibit water loss from evaporation. The experimental design is illustrated in Figure 3.8.

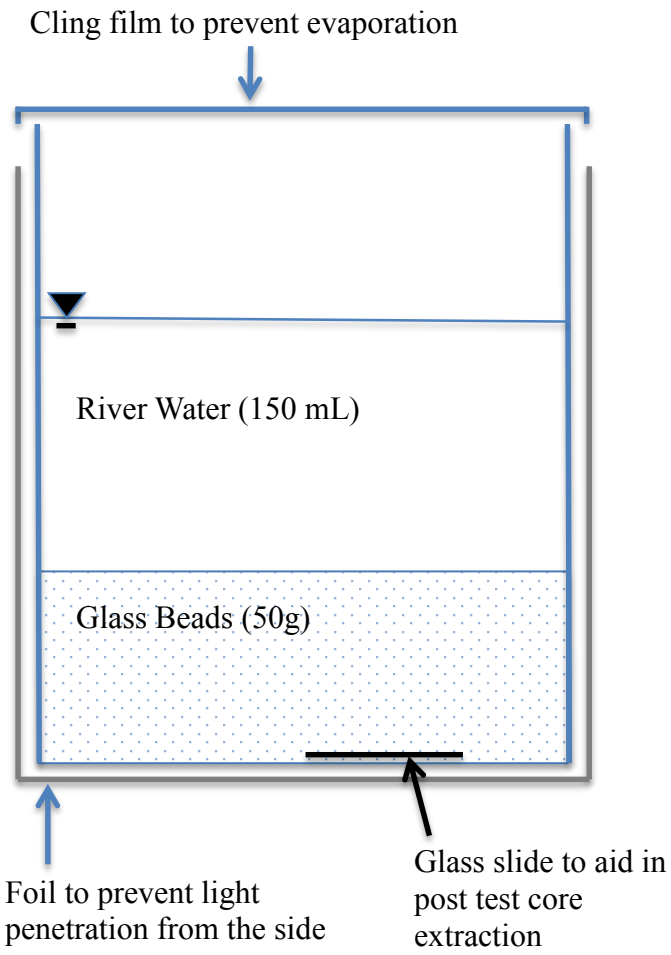


Figure 3.8: Experimental set up of homogenous sediment experiment to assess impact of sediment particle size on biofilm development

The experiment was run in a controlled environment room for a 10-day period, with an 18-hour light (Fluorescent daylight bulb with a Lee 226 filter) and six-hour dark cycle. Treatments for each sediment particle size and controls were prepared in triplicate. The experimental ratio between the sediment (50 g) and the volume of river water (150 mL) matched that of Loffler et al. (2005) and conformed to OECD 308 protocols. The incubation temperature was maintained at 20 ±1 ˚C, and bottles were incubated under constant motion using a rotary shaker (MK V Shaker, L.A. Engineering Co., UK) at 50 rpm. Following the 10-day experimental duration, the samples were photographed and cores were taken to permit the quantification of biofilm levels at different depths.

3.3.3.3 Microbial Analysis

The extracted cores were frozen and then segmented by depth at 0.5 cm intervals using a scalpel. Each 0.5 cm segment was placed into a 25 mL Falcon tube with 5 mL of water and vortexed to suspend the biofilm. Following this, 2 mL of suspended biofilm biomass was transferred to a clean test tube and 1 mL of 5% aqueous phenol (wt/vol) and 5 mL of concentrated H_2SO_4 were added to the solution. The tubes were then left to stand for 10 minutes, shaken, and then placed in a water bath at 30˚C for 20 minutes before absorbance was measured at 458 nm (see Dubois *et al.*, 1956). Carbohydrate content, which is indicative of microbial development due to their secretion by EPS, was calculated using the linear regression assay for glucose standards prepared in the range of 0 – 500 µg mL^{-1}.

3.3.4 Quantify the impact of biofilm on the rate of exchange as a result of clogging of interstitial pore spaces in flat bed systems

The impact of biofilm development on hyporheic exchange in different sediment particle size bed-forms required the implementation of an experimental design that would permit quantification of biofilm development over time, whilst assessing its impact on exchange. To do this, a 30-day experimental tracer test was conducted in a controlled environment room.

3.3.4.1 Sampling Procedure and Techniques

Water was collected from the River Dene in August 2013, as described in Section 3.3.3.2.

3.3.4.2 Experimental Set Up

3.3.4.2.1 Hyporheic Exchange Tests

The experimental series was set up in the same manner as described in Chapter 3, Section 3.2.1.2. A total of 6 flumes were used; triplicate replicates of flat bed hyporheic exchange tests using 2 different sediment particle sizes (5 mm and 0.5 mm). The different sized glass beads were used as a synthetic substrate in flume systems at a 10 cm depth with re-circulating river water from the River Dene (Chapter 3, Section 3.2.3.1) as inoculum to permit microbial development in the system under fluorescent light. However, modifications were made to this experimental design to permit the quantification of microbial development within each experimental unit; the addition of Perspex plates beneath bed material to aid in core extraction and 2.5 cm glass slides on the surface to act as extractable synthetic surfaces to use for biofilm quantification (see 3.2.4.2.2).

Repeat discrete injections of the tracer (Rhodamine WT) were added to each flume at 48-hour intervals to assess temporal variation in exchange over the 30-

day experimental period. Previous experimentation identified that repeat discrete injections on the same experimental system shared similar mixing rates within the water column, irrespective of time of addition, as described in Section 2.2. This ensured that the dosing conditions were comparable in duration and frequency irrespective of the time of administration and base level concentration.

3.3.4.2.2 Microbial Community Surface Development

Quantification of biofilm development in the flume systems was difficult, as any disruption to the sediment bed would have potentially altered the flow dynamics within the experimental unit. This could have impacted the bed-form and, in-turn, altered the rate of hyporheic exchange.

Similarly to other biofilm research (Kowalczyk et al., 2013), glass slides (2.5 cm^2 area) were added to the flume channels to act as an extractable surface to measure biofilm growth. Glass slides were situated, in triplicate, along the length of the flume bed surface at Time 0. These slides were distributed at 20 cm intervals along the sediment bed (Figure 3.9) and were found to have no impact on the rate hyporheic exchange (Chapter 2). During experimentation 3 slides were extracted every 48 hours from the same 2 locations on each experimental unit to assess biofilm development rate on the surface of the sediment bed.

Biofilm was scraped from each slide into 5 mL of deionised H2O using a scalpel, and total carbohydrate was measured as described in section 3.3.3.4. Visual analysis of biofilm development was performed using photographs of the test systems.

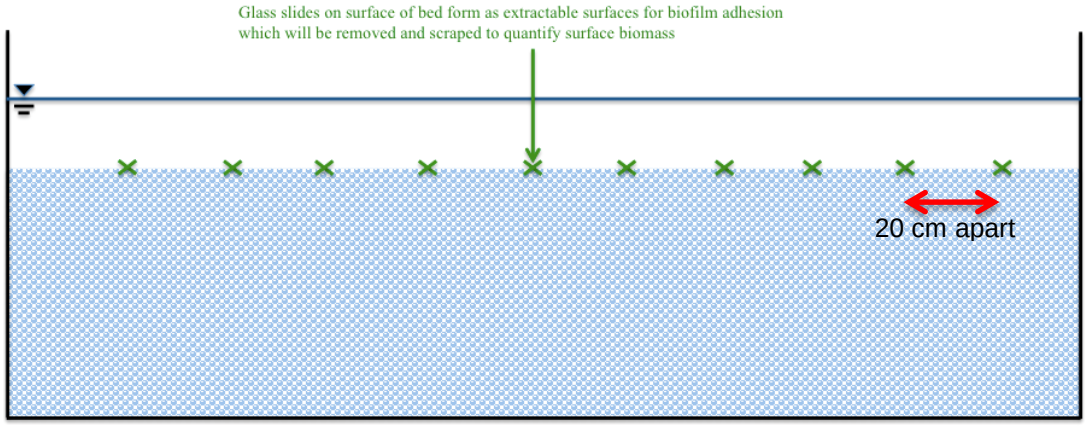


Figure 3.9: Placement of extractable glass slides on the sediment surface

3.3.4.2.3 Microbial Community Depth Penetration

Quantification of microbial development through the sediment bed itself could only be conducted at the end of the test, as examination of the sediment bed involved destructive sampling. To quantify the effective depth penetration of the microbial community through the sediment bed, coring was undertaken, based on the previous work described in Chapter 3, Section 3.2.3.

At the end of the test period, the water was drained from the test units and sediment cores were taken every 20 cm along the channel length to obtain an estimate of microbial development at depth in the different sediment beds. Perspex corers (length 10 cm; diameter 2 cm) were used to extract sediment, as illustrated in Figure 3.10. Once removed, the cores were dissected into sections at 1 cm intervals and analysed in the same manner as those described in Chapter 3, Section 3.2.3.4.

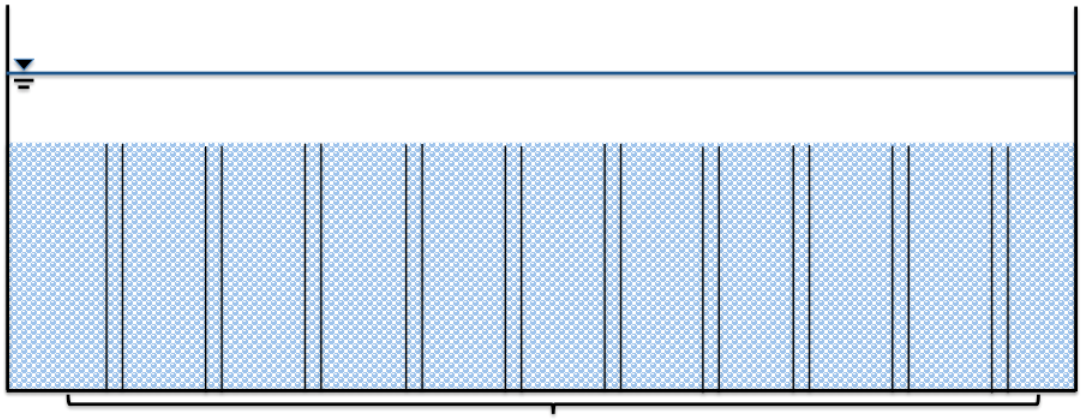


Figure 3.10a: Schematic of sediment coring to quantify effective depth penetration of microbial development from the bed material at Time End

Figure 3.10b: Example of a sediment coring of bed material at Time End

3.3.4.2.4 Water Chemistry Tests

To quantify microbial development in the flume systems, residual NO_3^+ and PO_4^- levels were monitored as a proxy for microbial growth (Costerton *et al.*, 1995). Water samples (5 mL) were taken from the weir end of each flume system at 48-hour intervals to avoid disturbing flow conditions. The water samples were then analysed using HACH testing kits to quantify the nutrient levels.

Moreover, the findings from the water chemistry tests permitted temporal monitoring of nutrient levels within the water column, particularly the nitrate and phosphate content. This was of particular importance as a reduction in these levels will result in a depletion of available nutrients for microbial biofilm development. As such, when the daily levels of nutrients were recorded as falling below 5 μg/ml, sodium nitrate and sodium phosphate were added to increase the availability of nutrients, thereby removing nutrient availability as a limiting factor in microbial community development.

These processes and the quantification of the nutrient dosing are described in Appendix A.

3.3.4.3 Trace Data Analytical Techniques

A data analysis method was devised to generate a standard reproducible method of start point identification from which exchange gradients could be quantified for utilisation in O'Connor and Harvey's (2008) hyporheic exchange calculation. To do this a computer programme was generated to conduct identical analysis on all datasets, eliminating subjectivity (Section 2.5).

3.3.5 Identify whether the wavelength of dunes affects biofilm development and hyporheic exchange

The impact of bed-form wavelengths on biofilm development and hyporheic exchange was studied.

3.3.5.1 Sampling Procedure and Techniques

Water was collected from the River Dene in November 2013, as described in Section 3.3.3.2.

3.3.5.2 Experimental Design

The experimental design described in Chapter 3, Section 3.2.4 was repeated. However, in this experimental series the impact of biofilm development on hyporheic exchange was investigated on two different undulating bed-forms rather than a flat bed-form. Wooden templates were used to generate reproducible desired bed-forms (λ=1 m, Δ=0.5 cm and λ=0.20 m, Δ=0.5 cm). These tests were conducted in triplicate for each bed-form on two different sediment particle sizes (5 mm and 0.5 mm). Water chemistry and biomass analyses were conducted at 48-hour intervals. Discrete tracer injections to quantify hyporheic exchange also took place at 48-hour intervals. $Time^{end}$ sediment cores were taken to determine the depth profile of microbial development between the differing sediment particle sizes and the different undulating bed-forms. This experimental design is illustrated in Figure 3.11.

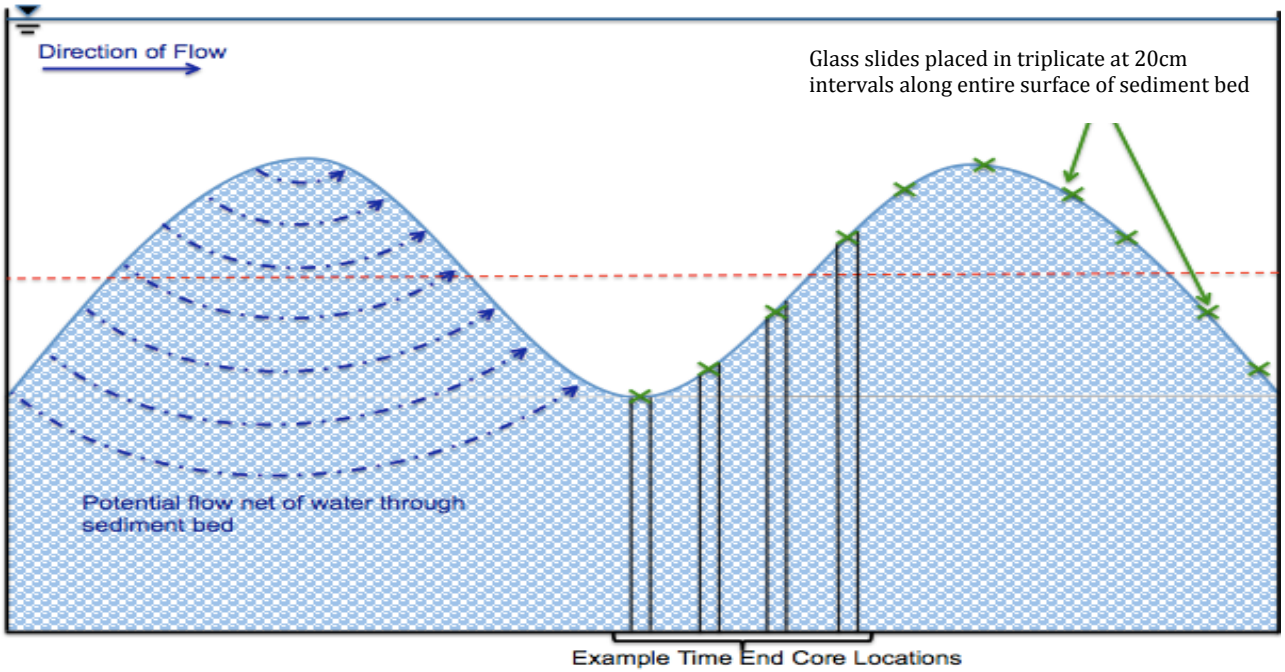


Figure 3.11: Experimental design to quantify microbial development at the surface and at depth in undulating dune systems; quantifying the impact of microbial development

3.3.6 Statistical Analysis

3.3.6.1 Analysis of hyporheic exchange rates

Analysis of variance (ANOVA) was used to identify the significance of differences ($P<0.05$) in rates of hyporheic exchange between the different sediment bed-forms and bed material particle sizes. All statistical analyses were performed using GenStat (13th edition, VSN International Ltd.). Analysis of parallelism, which is a form of linear regression that is used to assess groups, was also conducted on the calculated exchange coefficients. This was done to identify any significant difference in the slopes and intercepts recorded between treatment variables and within treatments (which may be different due to temporal variation).

Analysis of parallelism performed using GenStat (13th edition, VSN International Ltd.) required specification of the factor defining the different treatment groups, in this instance, either sediment particle size or bed-form depending on the analysis. For analysis of parallelism, three stages of analysis were undertaken. Initially, a primary model was fitted which ignored the groupings (simple linear regression) to identify whether the variance in the intercepts were significantly different at Time 0. Following this, the model was extended to include a different constant (or intercept) for each group, giving a set of parallel lines, one for each group. Then, the final model had both a different constant and a different regression coefficient (or slope) for each group. This analysis assesses the temporal variation in hyporheic exchange calculations, providing a point-by-point comparison of exchange values to quantify the difference between treatments and within treatments, with a confidence interval of 95 % (GenStat, 13th edition, VSN International Ltd.)

3.3.6.2 Statistical analysis of total carbohydrate data

Analysis of variance (ANOVA) was used to identify the significance of differences ($P<0.05$) in total carbohydrate levels between sediment particle sizes and with depths. Subsequently, a Tukey Post Hoc test was conducted to identify whether any of the sediment particle sizes significantly differed from each other.

3.3.6.3 Biofilm and Water Chemistry Analysis

Analysis of variance (ANOVA) was used to determine the significance of differences ($P<0.05$) in rates of nutrient depletion and biofilm development between sediment particle sizes and over time. Multivariate Analysis of Variance (MANOVA) was then conducted to ascertain whether the presence of biofilm communities impacted hyporheic exchange.

3.4 Results

3.4.1 Impact of sediment particle size on hyporheic exchange

In all replicates of flat bed hyporheic exchange, the systems that employed 5 mm synthetic glass beads as the porous media experienced a faster rate of exchange than the 0.5 mm sediment particle size. This is illustrated in Figure 3.12, which demonstrates the difference in the normalised rate of exchange between the different sediment systems. Figure 3.12 illustrates that the 5 mm sediment test reaches a level of equilibrium after 140 seconds$^{0.5}$, at which point the 0.5 mm sediment system is still exchanging at a slower rate. The difference in exchange is best seen in the mean hyporheic exchange gradients, with the 5 mm sediment exchange faster with a gradient of -0.0052 (± 0.000259) compared to the 0.5 mm sediment, which had a mean gradient of -0.0034 (±0.000723). The recorded differences in the comparative rates of hyporheic exchange on the varying sediments was identified as significant using ANOVA ($P<0.001$).

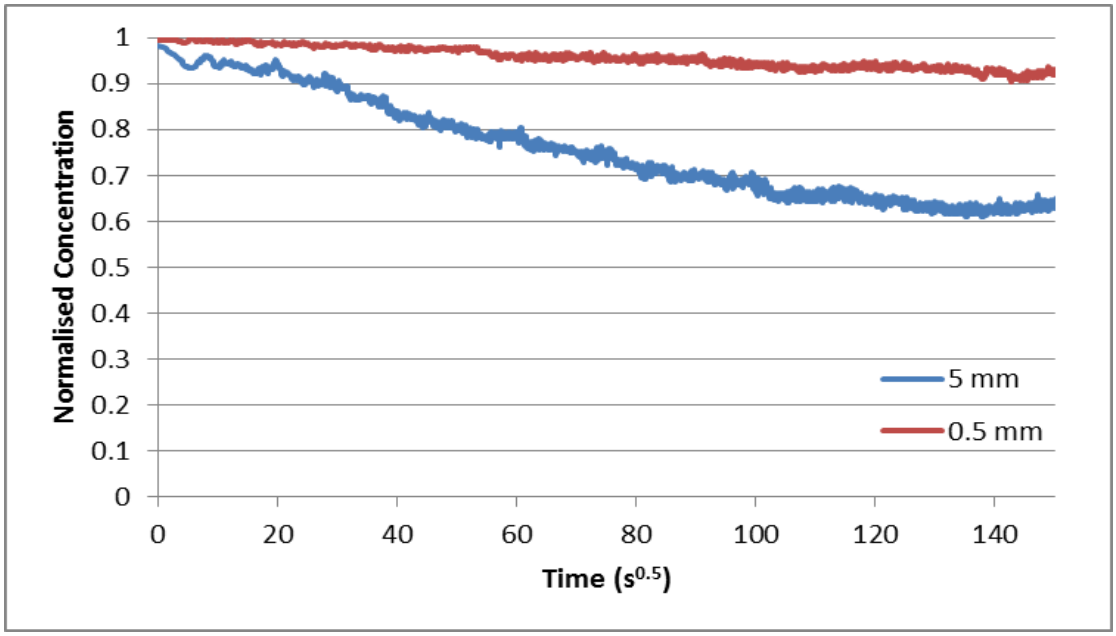


Figure 3.12: Example comparative normalised rate of exchange in the experimental systems following initial dye injection at Time 0

3.4.2 Impact of bed-form morphology on hyporheic exchange

Trace tests conducted in the flume system recorded different rates of exchange between different bed-forms (Figure 3.13). There was a rapid rate of mixing following injection in the undulating bed system, with a plateau level reached before 5000 seconds of testing elapsed. Conversely, there was a more gradual rate of exchange in the flat bed system, shown by a more gradual decline in concentration over time and a plateau level reached closer to 15000 seconds (Figure 3.13). Normalised concentration analysis also showed the same trend; a faster rate of exchange in the undulating bed system (mean gradient of -0.005153 ± 0.000723) when compared to the mean flat bed gradient (mean gradient of -0.004834 ± 0.00068), seen in the steeper gradient prior to reaching equilibrium levels (Figure 3.14). ANOVA identified a significant difference in the rate of exchange between the treatments ($P<0.001$).

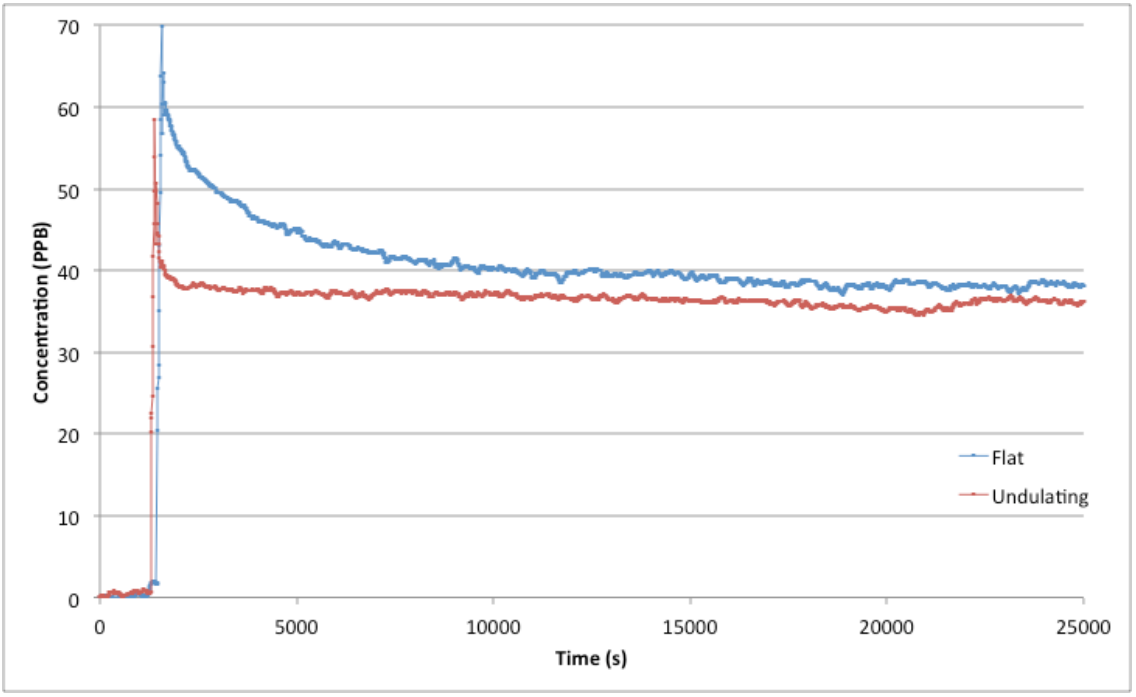


Figure 3.13: Example comparative rate of exchange in the experimental systems following Rhodamine tracer injection on 5mm sediment

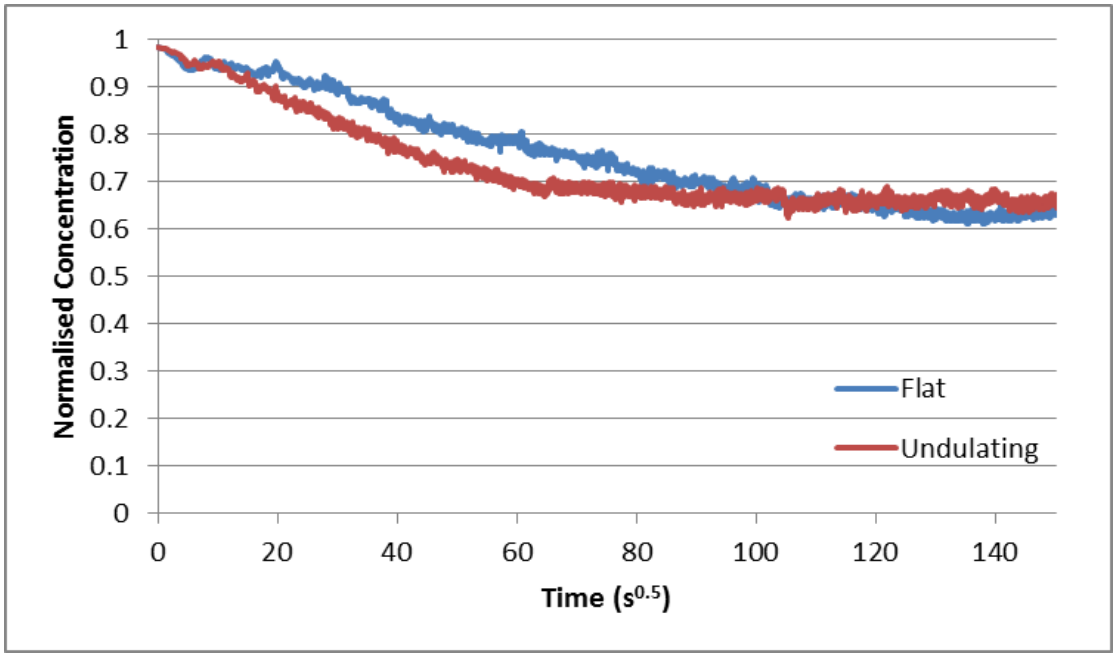


Figure 3.14: Comparative rate of exchange in the experimental systems (5 mm sediment particle size)

3.4.3 Microbial development on different sediment particle sizes

Visual analysis of the beaker experiments indicated microbial community development disparities between the experimental treatments (Figure 3.15). It was found that the surface layer of the experimental units containing 0.5 - 0.75 mm, 0.3 - 0.4 mm and 0.1 - 0.2 mm sediments had a visibly higher accumulation of biofilm than at depth. The larger particle systems (5 mm and 1.7 mm) were found to have seemingly uniform development of biofilm around each independent particle, irrespective of depth (Figure 3.15). Biomass quantification supported this visual analysis, indicating that the biomass levels were concentrated at the surface of the smaller sediment particle sizes 0.5-0.75 mm, 0.3 - 0.4 mm and 0.1 - 0.2 mm sediments), whereas there was a more even distribution throughout the larger sediment particle sizes (5 mm and 1.7-2.1 mm). This is illustrated in Table 3.2, which demonstrates a reduction in recorded carbohydrate levels with depth in each experimental unit, with a smaller reduction seen in those experiments containing 5 mm and 1.7 mm. However, Table 3.3 which shows the carbohydrate area density around the

Table 3.2: Comparison of biofilm development on different sized glass beads at depth in a closed, *ex-situ* system

Mean sediment particle size (mm)	Carbohydrate concentration at different depths from sediment surface (mg/cm^3)			
	0 – 0.5 cm	0.5 – 1.0 cm	1.0 – 1.5 cm	1.5 – 2.0 cm
5	86.6[a]	69.1 [a]	71.5 [a]	64.3 [a]
1.9	88.9 [a]	84.2 [a]	75 [a]	58.9 [a]
0.625	102.9 [b]	84.2 [b]	82 [b]	35.2 [b]
0.35	119.2 [b]	100.3 [b]	83.8 [b]	28.7 [b]
0.15	118.2 [b]	95.7 [b]	72 [b]	33.2 [b]

Table 3.3: Comparison of the amount of the surface area of carbohydrate on different sized sediments

Sediment size (mm)	Surface Area Sphere (cm^2)	Carbohydrate area density at different depths (mg/cm^2)			
		0 – 0.5 cm	0.5 – 1.0 cm	1.0 – 1.5 cm	1.5 – 2.0 cm
5	4.7	6.01	4.80	4.97	4.47
1.9	12.4	2.79	2.64	2.35	1.85
0.63	37.4	1.08	0.88	0.86	0.37
0.35	67.3	0.70	0.59	0.49	0.17
0.15	157.1	0.30	0.24	0.18	0.083

Carbohydrate (mg/cm^2) is calculated based on the assumption that the volume remaining from the volume core-volume of sediment is completely full of carbohydrate (Carbohydrate = vol core - vol sphere). This is the maximum possible carbohydrate that could be in the section at any time. The sum of the carbohydrate on all spheres is found by calculating the surface area of a sphere and by determining the amount of spheres in the section to get total surface area that can be covered by carbohydrate. The total carbohydrate (mg) is divided by surface area to quantify the maximum amount that is present on the surface of the spheres in each section.

spheres, shows that there is a reduction in carbohydrate level with depth in all experimental units, irrespective of sediment particle size. Table 3.3 also shows that there is a significant difference between the surface level carbohydrate presence on all sediment particle sizes, with 5 mm sediments possessing the highest and the amount reducing with decrease in sediment particle size. Additionally, there is a significant difference between all surface level

carbohydrate levels (0-0.5 cm) and those at greatest depth (1.5-2.0 cm), suggesting that microbial development is not uniform through sediments, potentially attributable to light availability.

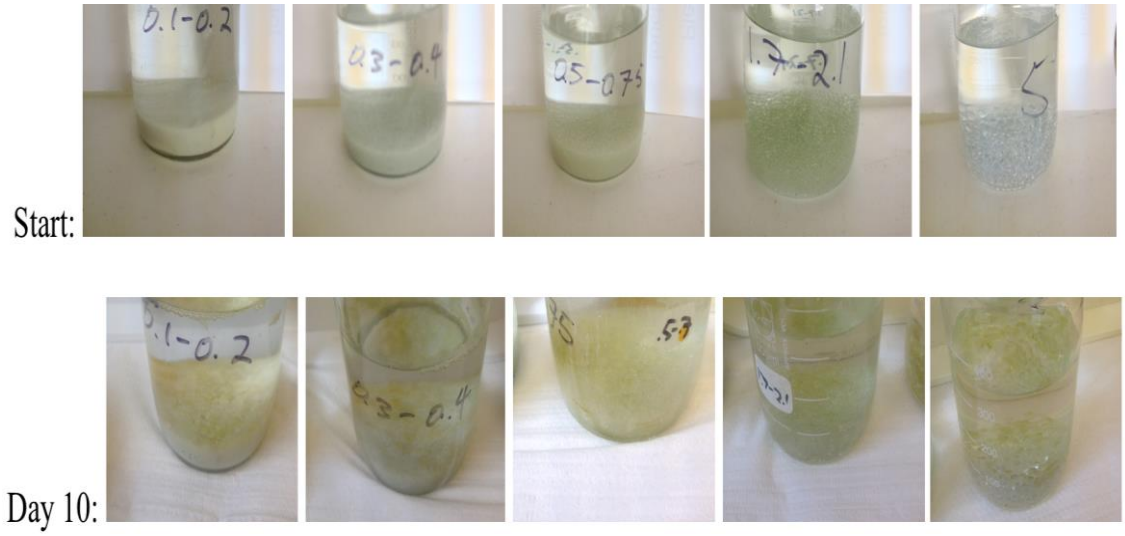


Figure 3.15a: Time 0 and Time End visual comparison of biofilm development on different sized glass beads

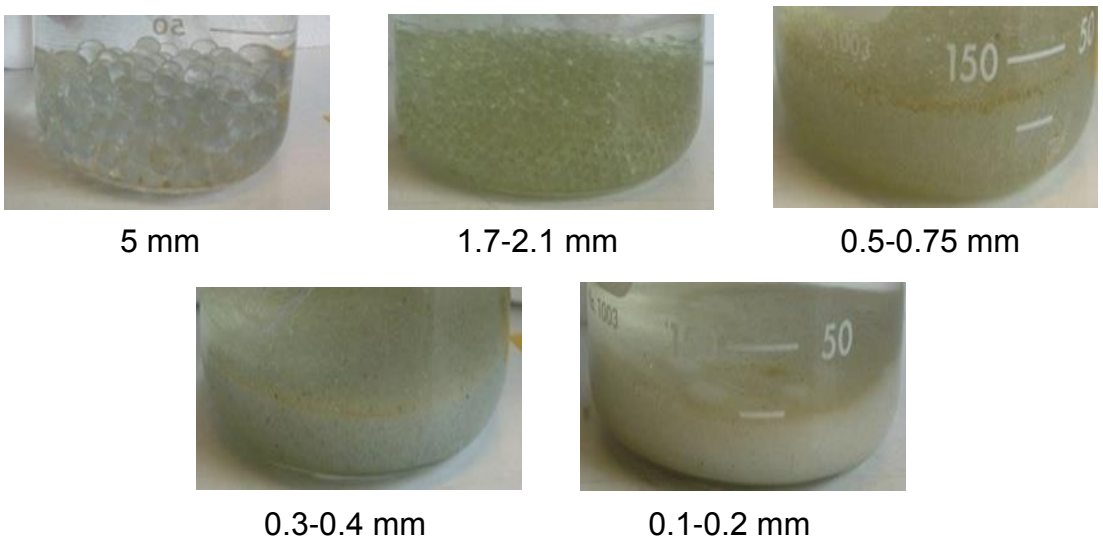


Figure 3.15b: Side View of Time End visual comparison of biofilm development on different sized glass beads

sediment particles. ANOVA indicated that there was a significant reduction in biomass with increased depth from surface, irrespective of sediment particle size ($P < 0.001$). Tukey post-hoc analysis indicated that there was a significant difference between samples. Sediments sized 0.1 mm, 0.3 mm and 0.5 mm possessed a significantly different biofilm depth distribution than the 5 mm and 1.7 mm sediments ($P < 0.001$), with biofilm concentrated at surface.

3.4.4 Microbial development in flat bed re-circulating flume systems using different sediment particle sizes

The depth distribution patterns of the biofilm varied between the two sediment particle sizes (Figures 3.16, 3.17 and 3.18). Figure 3.16 shows that biofilm community development occurred in all experimental units. Figure 3.16 also indicates that biofilm community development penetrated to greater depths within the sediment bed in the treatments employing 5 mm particle sizes compared to 0.5 mm experimental systems, in which biofilm development concentrated at the surface of the sediment, as a mat. Figure 3.17 supports this, illustrating that biofilm development occurred in all systems, which gradually increased over the course of the test until the biomass levels plateaued after 13 days. This indicates similar temporal growth rates on the sediment surfaces of the different experimental treatments. However, Figure 3.17 shows a higher level of biomass on the sediment surface of the 0.5 mm sediment particles.

Differences in biofilm community development in the different experimental treatments are illustrated in Figure 3.18, where the recorded biomass levels taken from $Time^{End}$ cores differed significantly ($P>0.01$). Figure 3.18 shows the highest biomass levels are recorded at the surface of both experimental treatments. However, in the 5 mm sediment systems, there is a gradual reduction in biomass levels with depth. Conversely, the 0.5 mm sediment systems show a rapid reduction in biomass levels after the initial 2 cm depth. This shows that the biomass levels were primarily located on the surface of the 0.5 mm sediment bed, whereas higher levels were found to have infiltrated in abundance to greater depths in the 5 mm sediment system, with a gradual reduction in concentration level at depth.

Figure 3.16a: Biofilm development on the surface of different bed materials in re-circulating flume system

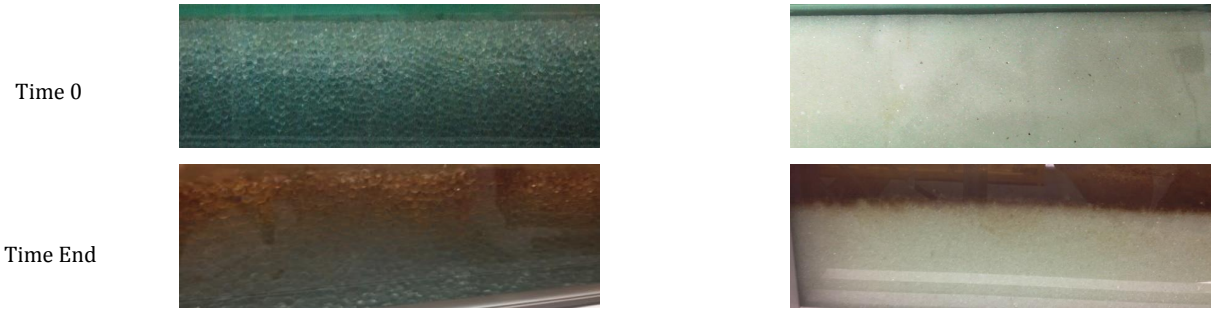


Figure 3.16b: Microbial community depth development on different bed materials in re-circulating flume system

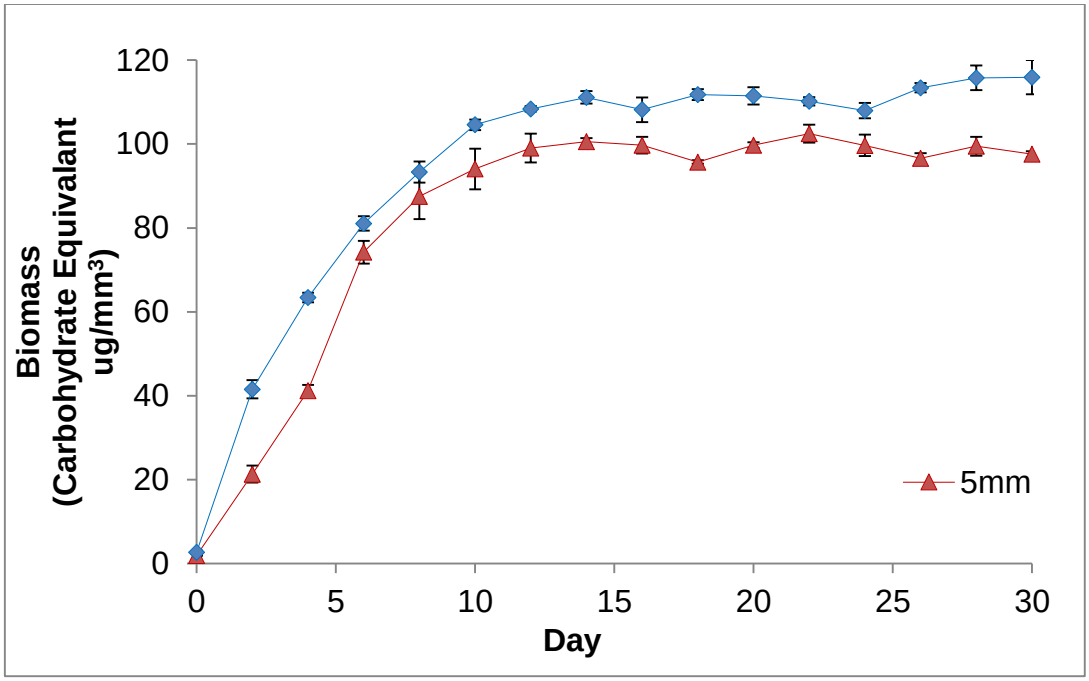


Figure 3.17: Flat Bed microbial community development on sediment surface in flumes of different sediment particle sizes. Error bars represent ± standard error of the mean

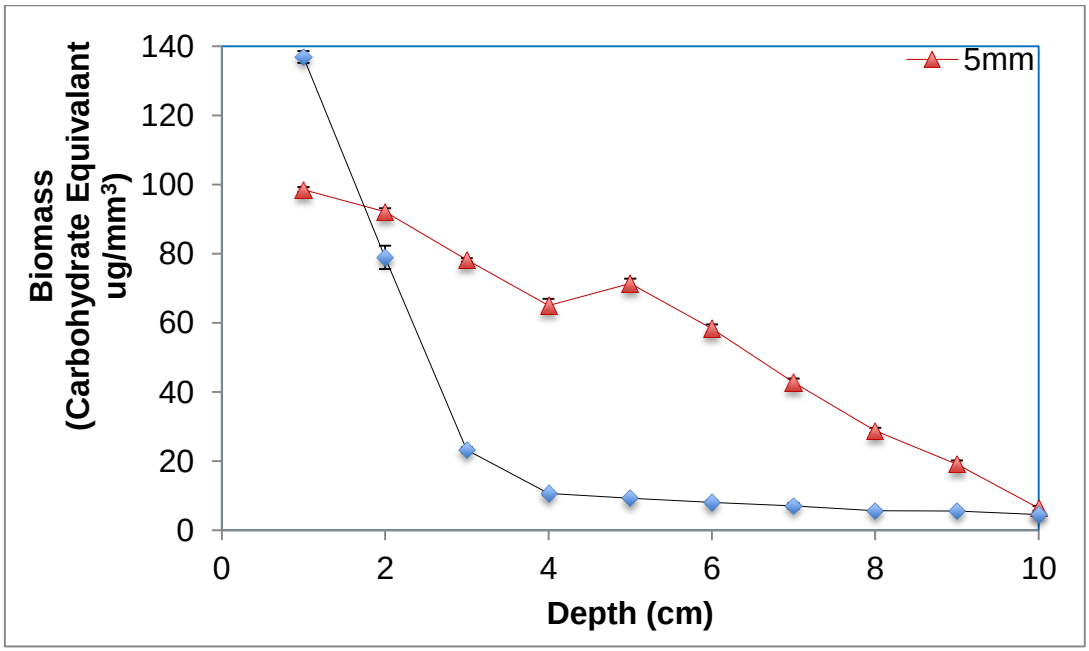


Figure 3.18: Microbial community depth penetration quantified from Time^{End} cores. Error bars represent ± standard error of the mean

3.4.5 Effect of particle size and microbial development on hyporheic exchange in flat bed re-circulating flume systems

Figure 3.19 illustrates an example of temporal variance in normalised hyporheic exchange rates following bi-daily repeat discrete tracer injections. This figure shows a gradual reduction in exchange gradient over time, with a lessening of the gradient with each additional day.

ANOVAs indicated a significant difference in the rate of exchange between the two sediment particle sizes at Time 0 ($P=0.034$), with a faster rate of exchange occurring in the 5 mm particle size system than the 0.5 mm sediment system (Figure 3.20). Figure 3.20 shows the analysis of parallelism conducted on the exchange gradients over the duration of the experiment, which is based on identification of significant difference between the lines of best fit for the datasets of the treatments being analysed. Figure 3.20 illustrates a significant reduction in exchange gradients in the 0.5 mm sediment tests over time ($p<0.001$), but no significant change in the 5 mm sediment tests ($P=0.222$). Additionally, repeated measures ANOVA indicated that the temporal variation in hyporheic exchange recorded in the 0.5 mm sediment particle size was significant ($P<0.001$). This suggests that the rate of exchange reduces significantly in the 0.5 mm sediment over time, with no significant temporal variation recorded in the 5 mm sediment system.

3.4.6 Impact of bed-form wavelength on microbial development and its subsequent effect on hyporheic exchange

Microbial development was measured on the surface and at depth in the 2-dune and multiple dune experimental systems. Over the course of the tests there were visual differences in microbial development with different sediment particle sizes and different bed-forms (Figures 3.21, 3.22, 3.23 and 3.24). These images show that the majority of the visible microbial development appeared to concentrate on the surface of the 0.5 mm sediment particle size as a mat, whereas the 5 mm particles permitted microbial development throughout the sediment bed irrespective of bed-form.

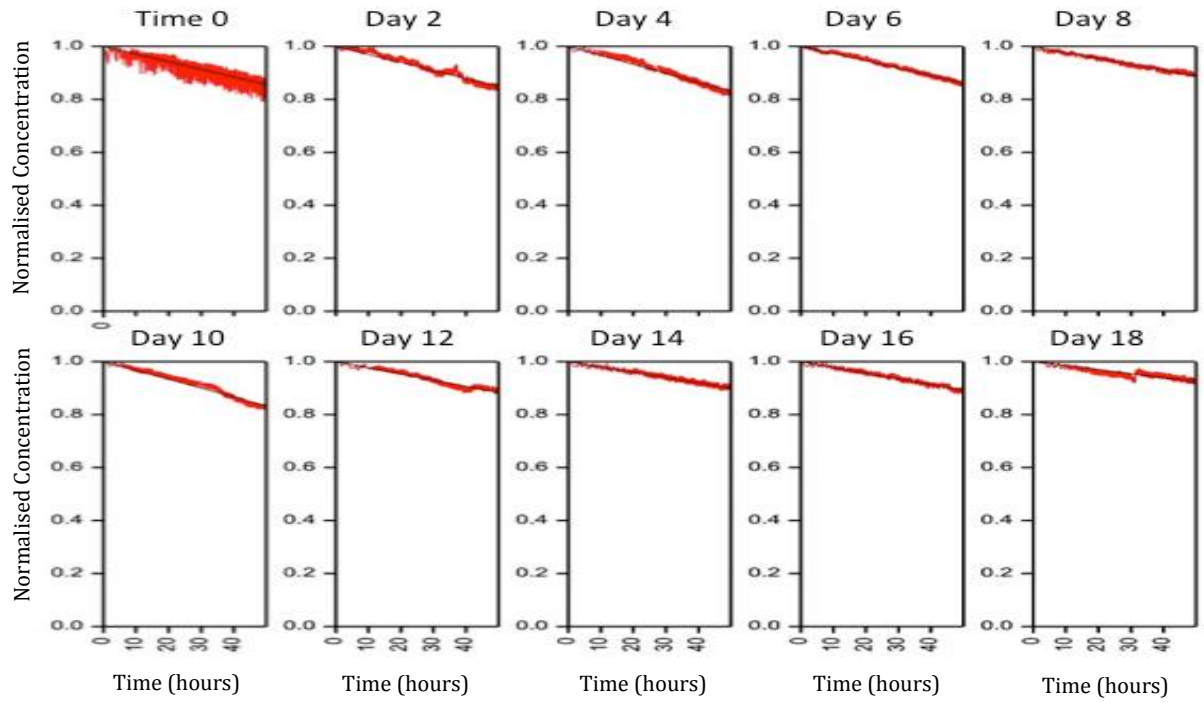


Figure 3.19: Example of temporal variance in normalised hyporheic exchange rates following bi-daily repeat discrete tracer injections

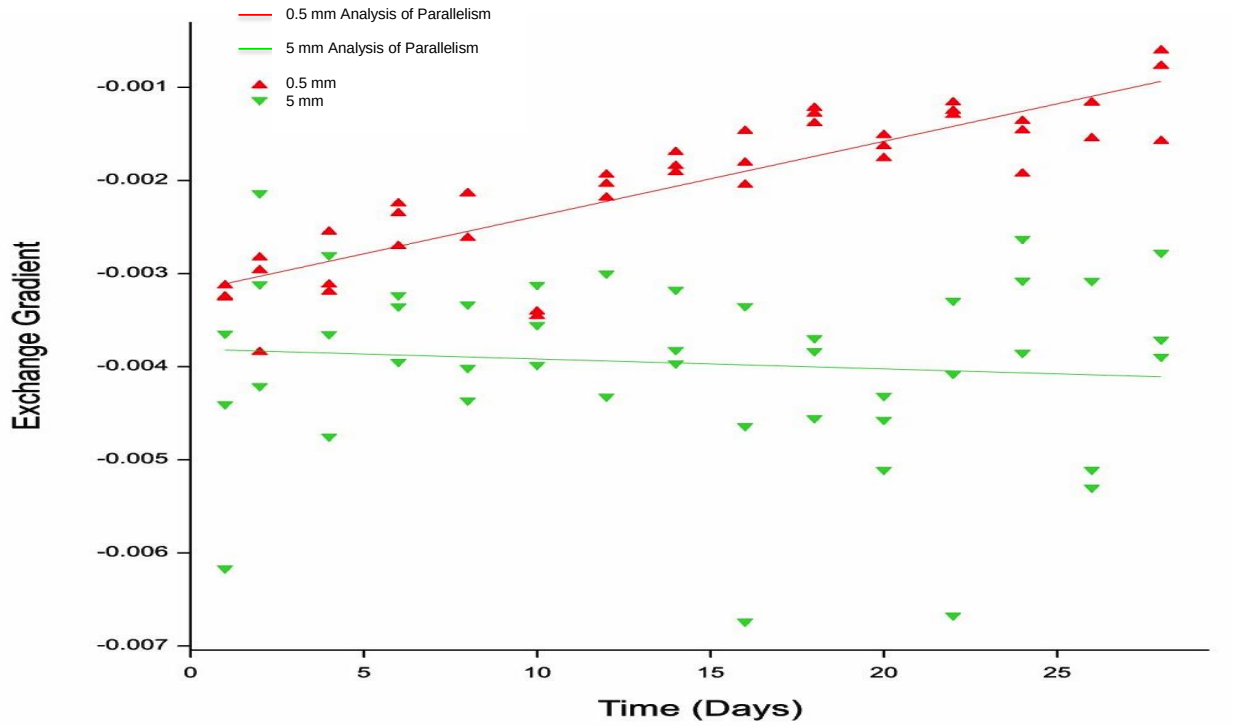


Figure 3.20: Temporal variance in normalised hyporheic exchange gradients from flat bed flume tests; taken at 30 % exchange

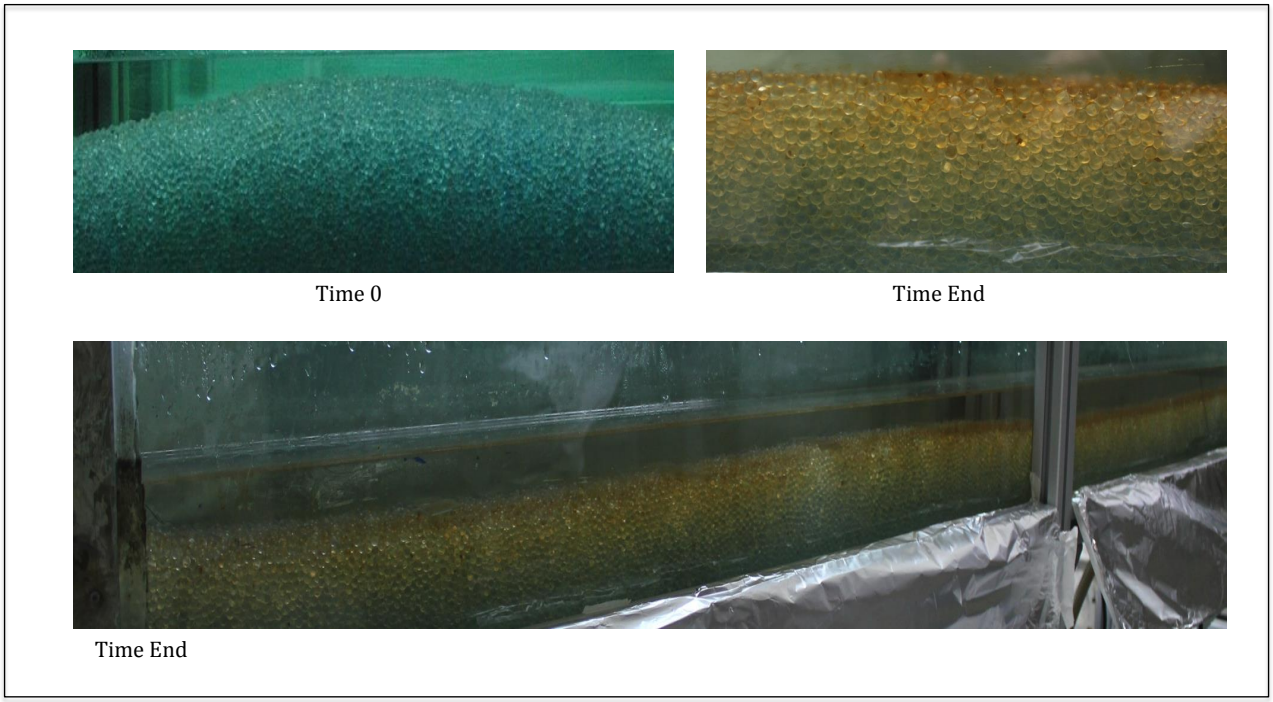


Figure 3.21: Example microbial community depth development on 5 mm glass beads in undulating (2-dune) flume system

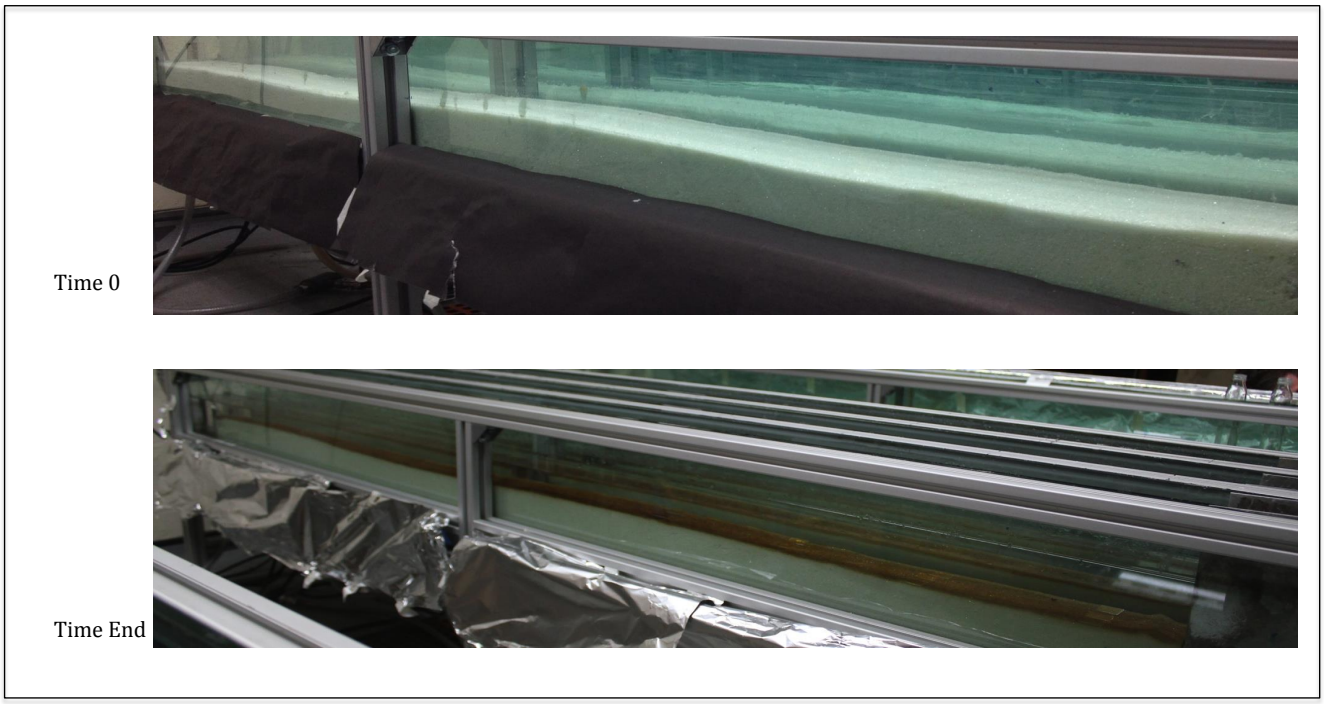
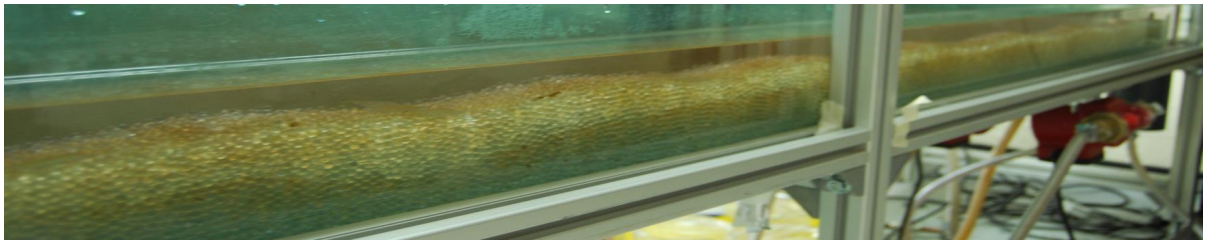


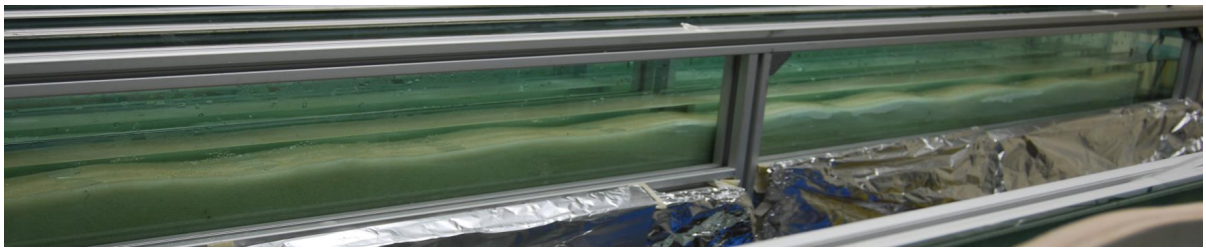
Figure 3.22: Example of microbial community depth development on 0.5 mm glass beads in undulating (2-dune) flume system

Time 0

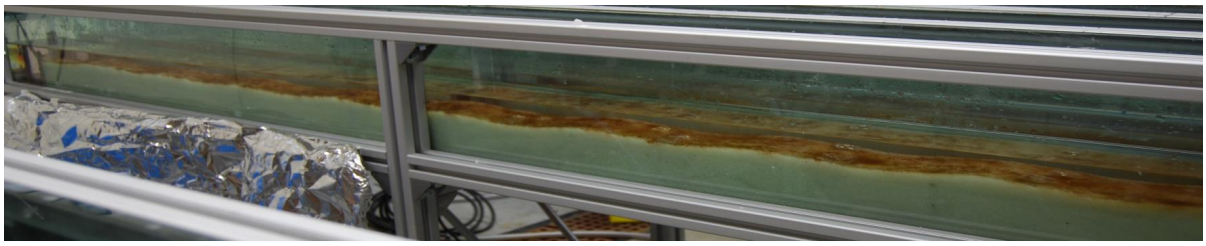


Time End

Figure 3.23: Example microbial community depth development on 5 mm glass beads in undulating (multiple) flume system



Time 0



Time End

Figure 3.24: Example microbial community depth development on 0.5 mm glass beads in undulating (multiple) flume system

Figure 3.25 shows differences between the biomass developments at depth in the varying bed-form tests, indicating different levels of biofilm depth infiltration in the multiple dune tests, dependent upon the part of the bed-form the core was extracted from. Figure 3.25 indicates a higher level of surface biofilm development in the 0.5 mm sediment systems than the 5 mm sediment systems. It shows a higher level of biomass at depth in the 5 mm sediments than the 0.5 mm sediments, resembling those differences seen in the flat bed tests (see Figure 3.17). Moreover, Figure 3.25 indicated that the 0.5 mm sediment tests which employed multiple dunes had higher levels of biomass development at depth at the peak, up-slope and down-slope sections of the system, compared to the higher levels of biomass recorded in the 2-Dune system at depth for the 0.5 mm sediment in the trough section. Figure 3.25 also shows that in the multiple dune system the 0.5 mm systems had a greater abundance of biofilm development at depth in the peak, up-slope and down-slope sections of the system, with higher levels of biofilm being recorded up to depths of 4 cm, compared to 3 cm in the 2-dune systems.

Figure 3.26 shows the variance in biofilm development at different locations at depth in the different dune systems. In keeping with the findings of Section 3.4.4, Figure 3.26 indicates a significantly higher level of biomass on the surface of the 0.5 mm sediment particle size than the 5 mm sediment particle size in both bed-form tests, which was supported by ANOVA ($P=0.027$).

To quantify whether or not the accumulation of microbial communities on the different bed-forms impacted hyporheic exchange, the trace data was normalised to permit identification of temporal changes in exchange gradients, illustrated in Figures 3.27 and 3.28. Figure 3.27 indicates the dynamics in exchange rates between different sediment particle sizes for an undulating bed-form test with two dunes. The figure illustrates a reduction in exchange in the 0.5 mm sediment particle size as the duration of the test continued, shown by the diminuation in the measured exchange gradient taken from the normalised data. Conversely, no difference in exchange over time was seen in the 5 mm sediment particle size. ANOVA supports this, identifying a significant relationship between time and exchange gradient in the 0.5 mm experimental

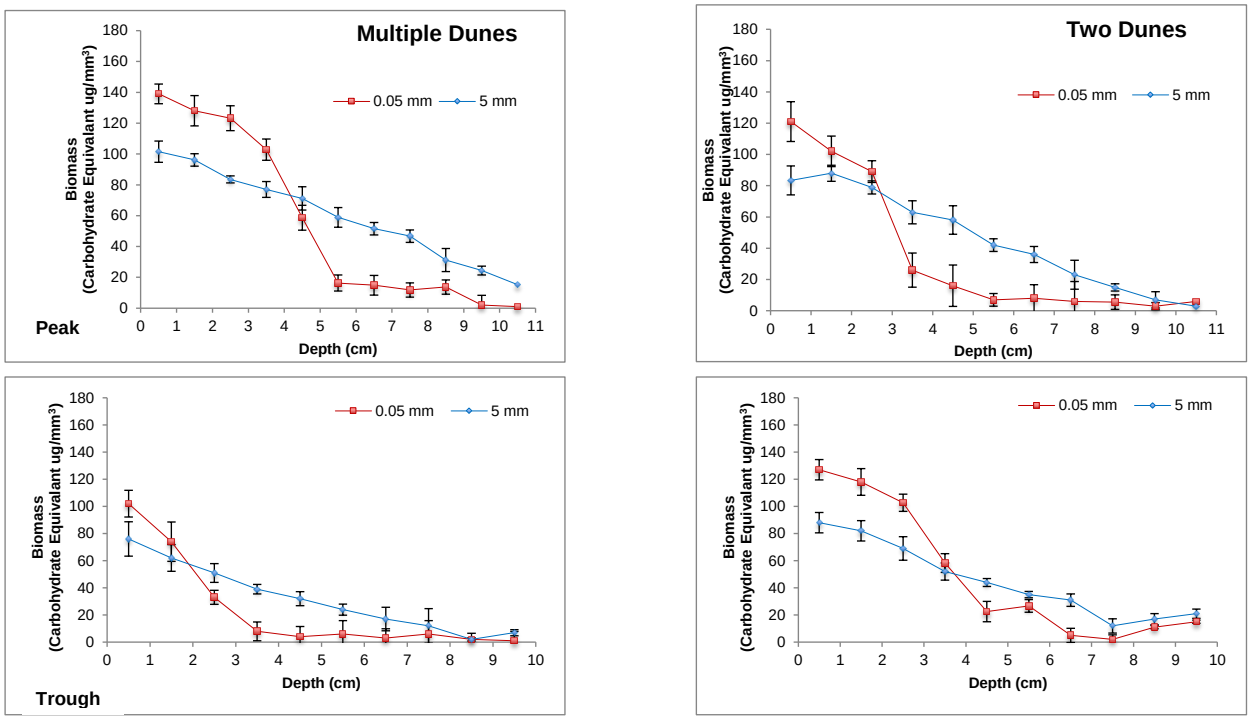


Figure 3.25a: Biomass levels at depth in dune tests. Error bars represent ± standard error of the mean

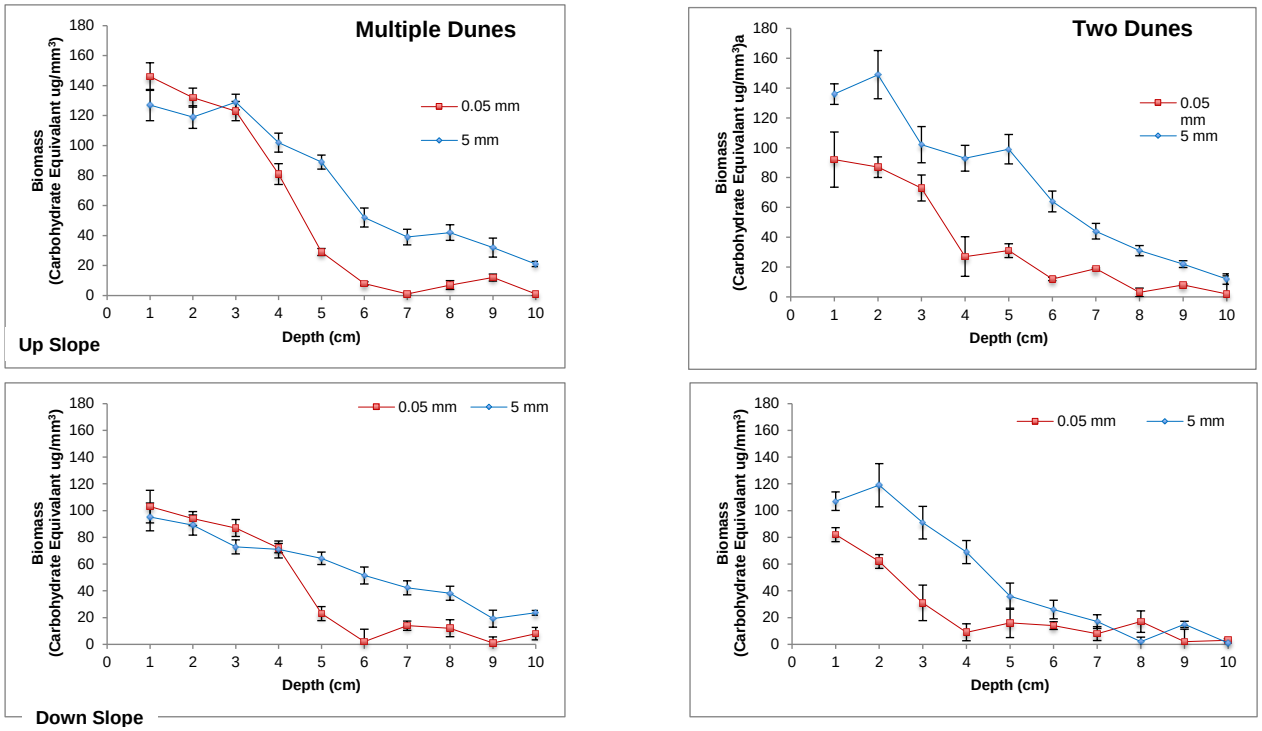


Figure 3.25b: Biomass levels at depth in dune tests. Error bars represent ± standard error of the mean

data (P=0.019) and no significant relationship for the 5mm sediment particle size (P=0.503). This trend was also observed in the multiple dune tests, with Figure 3.28 demonstrating the same trend of significant reduction in hyporheic exchange in the 0.5mm sediment tests and no significant reduction in hyporheic exchange in the 5mm sediment tests. This was confirmed with ANOVA (P=0.014; P=0.47).

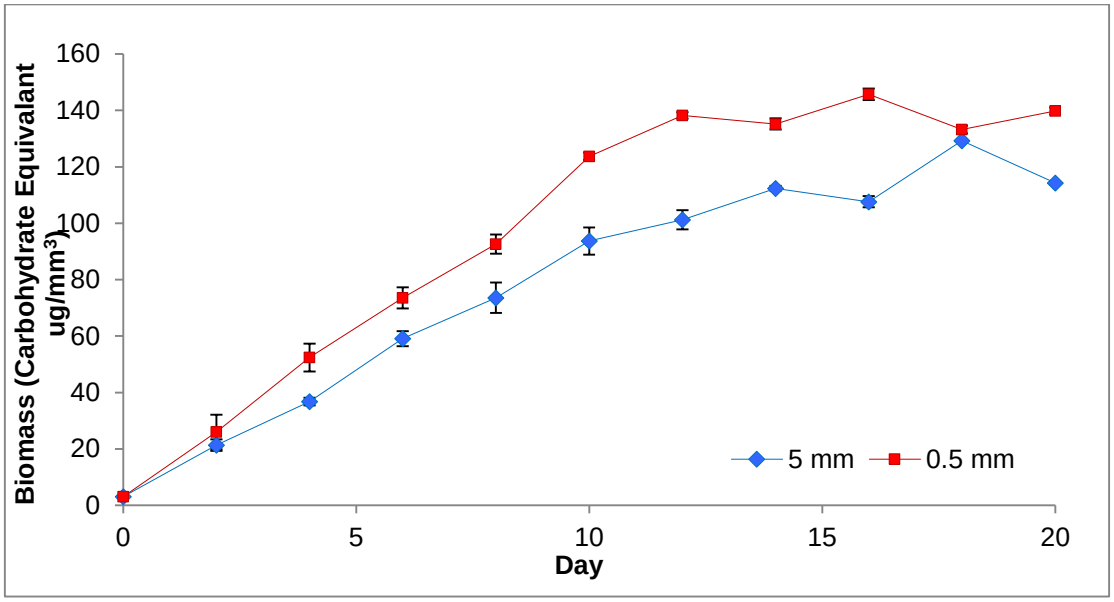


Figure 3.26a: Microbial development at surface in multiple dune tests. Error bars represent ± standard error of the mean

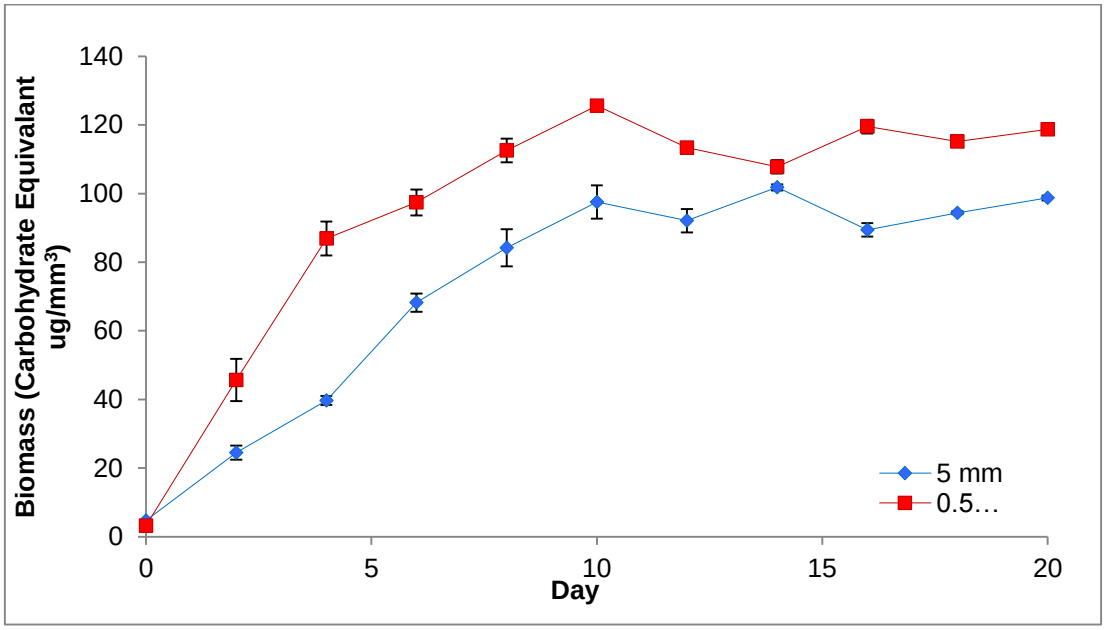


Figure 3.26b: Microbial development at surface in two dune tests. Error bars represent ± standard error of the mean

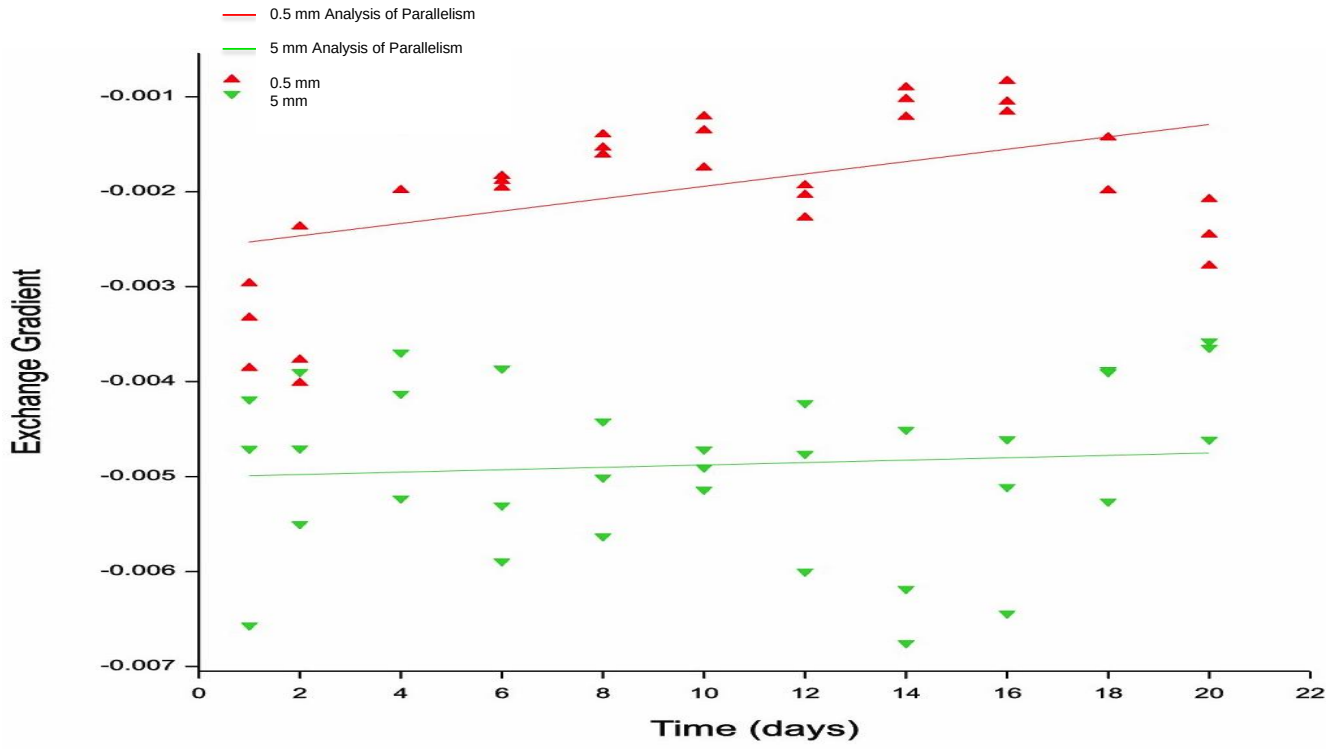


Figure 3.27: Variance in Normalised hyporheic exchange gradients from 2 undulating dune flume tests; 30 % exchange

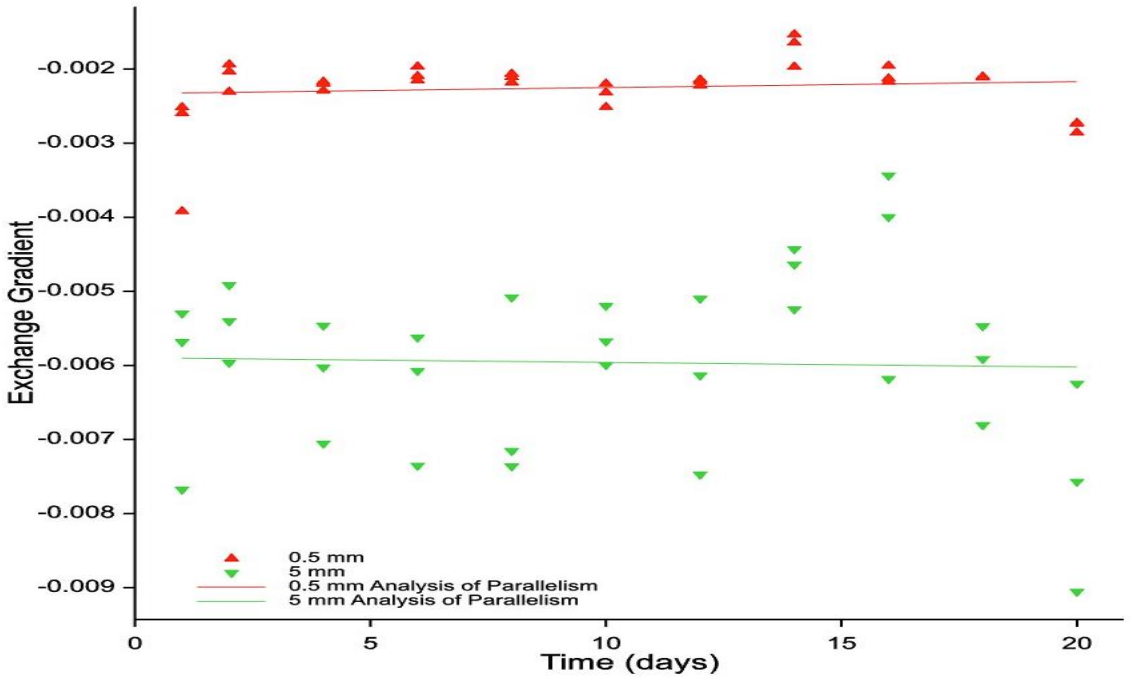


Figure 3.28: Variance in normalised hyporheic exchange gradients from multiple dunes flume tests; 30 % exchange

3.5 Discussion

The accuracy, reproducibility and repeatability of the experimental flume systems were supported by data obtained in current study. The hyporheic exchange tests conducted demonstrated a faster rate of exchange in sediment systems with larger sediment particle sizes and faster exchange in systems with bed-forms present, findings that are in-keeping with other flume studies due to the nature of hyporheic exchange (Elliott and Brookes, 1997a; O'Connor and Harvey, 2008). Utilising river water as inoculum in the hyporheic exchange tests provided an insight to the variance seen in microbial community development in sediment beds, with variance between the effective depth penetration of the biofilm in the sediment bed dependent upon the particle size and sediment bed-form. Additionally, this study enabled quantification of the impact of microbial community development on hyporheic exchange, identifying that the clogging of interstitial porous spaces of fine sediments by biofilms inhibits the rate of exchange, furthering the work of Nogaro *et al.,* 2010.

3.5.1 Effect of Sediment Size on Hyporheic Exchange in Experimental System

Trace test quantification confirmed variance in exchange due to sediment particle size, identifying the reproducibility of the experimental system and supporting the findings of investigative hyporheic exchange studies that have been employed in other flume studies (Elliot and Brookes, 1997; Marion et al., 2002; Dutton, 2004). Figure 3.12 illustrates an example of the difference in hyporheic exchange rate between two differing particle sizes, where a significant difference in exchange gradient was found dependent upon sediment particle size, with larger sediments resulting in faster rate of exchange ($p<0.01$). These findings were in-keeping with similar experimental studies that have identified hyporheic exchange occurring faster in larger sediments owing to the larger pore space within the sediment bed permitting easier transfer of fluid from the water column to the interstitial porous space within the sediment (Dutton, 2004; Chandler, 2013; Marion et al., 2008). This means that the novel

experimental system used in this study is capable of measuring hyporheic exchange, with the variance recorded between rates of exchange attributable to differences between sediment particle size in accordance with other experimental work (Richardson and Parr, 1988; Lai et al., 1994; Elliot and Brooks, 1997b; Tonina and Buffington, 2007).

3.5.2 Effect of Bed-Form on Hyporheic Exchange in Experimental System

Figures 3.13 and 3.14 are example traces (ppb and normalised) that demonstrate distinct differences in the rate of hyporheic exchange for different bed-form profiles. Figure 3.13 illustrates a rapid rate of mixing following tracer injection in the undulating bed system; with a plateau level reached before 5000 seconds of testing has elapsed. Conversely, there was a much slower rate of exchange in the flat bed system, suggesting a slower rate of hyporheic exchange in the experimental system due to the bed-form. This trend is supported by the normalised data (Figure 3.14), seen in the steeper gradient. The difference illustrated by the ANOVA ($P<0.01$) infers that the rates of hyporheic exchange are significantly different between the bed-forms, supporting literary expectations and further enforcing the suitability of the experimental systems for elucidating the difference in exchange rates between flat and undulating beds.

These findings are comparable to those found from methodologies employed in other flume studies (Elliot and Brookes, 1997; Marion et al., 2002; Dutton, 2004), with a faster rate of hyporheic exchange identified when bed-forms are present in the river system. The increases in rate of hyporheic exchange within river systems that possess bed-forms are attributable to pressure variations over the bed surface that cause interstitial flow through the bed surface and within the bed (Elliot and Brooks, 1997a). The quantification of this difference in exchange rate as a result of bed-form within the experimental system provided confidence in the experimental methodology as the results were as expected in accordance to expectations (Grimm and Fischer, 1984; Savant et al., 1987; Marion et al., 2002; O'Connor and Harvey, 2008). This means that the test systems are

suitable for quantifying the impact of microbial development on exchange rates as the systems respond in a similar manner to the natural riverine environment.

3.5.3 Effective Depth Penetration of Microbial Community on Differing Sediment Particle Sizes

Provisional experimentation suggested that microbial community development varied in quantity and location on sediment beds of different sized particles. To confirm and quantify this, experimentation was conducted to compare biomass and EPS development (reflective of microbial community) at different depths on varying sediment particle sizes in controlled experimental units via quantification of biomass. Figure 3.15 suggested differences in microbial community development and dispersion on alternate sediment particle sizes based on visual analysis. Quantification of the microbial community development indicated concentration on the surface layer of all particle sizes tested that were smaller than 0.75 mm. The larger particles had biofilm development around each independent particle irrespective of location and depth. This is potentially attributable to the ease of fluid movement around the larger sediment particle sizes, as the ease of movement from the surface to depth will enable nutrient movement and light passage through the sediment bed, thereby permitting biofilm development at greater depths and encompassing the larger sediments as independent particles as opposed to just over the surface layer.

Carbohydrate testing of extracted cores supported the visual analysis, indicating that the microbial development was concentrated at the surface of the smaller sediment particle sizes (<0.75 mm), whereas there was a more even distribution throughout the larger sediment particle sizes. ANOVA showed that microbial community development in the small sediments (<0.75 mm) was significantly different to those of a larger sediment particle size. Tukey post hoc analysis indicated that 0.1 mm, 0.3 mm and 0.5 mm sediments had different biofilm development at depth compared to 1.7 mm and 5.0 mm, with a smaller amount of glucose secreted by EPS found further within the sediment bed (Table 3.2). The more even distribution of biomass in the larger sediments

supports the theory that the availability of light and nutrients is higher at depth than in the smaller particle size, attributable to the larger interstitial porous spaces.

The variance in distribution of microbial community on different particle sizes implied that varying sediments might possess different infiltration capacities due to pore space variance in the sediment bed (Nogaro *et al.,* 2010). As limited work has been conducted to quantify or assess the impact of sediment clogging on hyporheic exchange, it was necessary to conduct this experiment in a lotic system.

3.5.4 Impact of Microbial Development on Hyporheic Exchange

3.5.4.1 Microbial Community Development on Different Sediment Particle Sizes in Flat-Bed Experimental system

Flat bed flume tests conducted in the re-circulating flume systems using two differing sediment particle sizes (0.5 mm and 5 mm) permitted temporal development of microbial communities on and within the sediment beds. Figure 3.16 illustrates the visual variance between the microbial development in the sediment systems, indicating a concentrated build up of microbial communities on the sediment surface of the 0.5 mm sediment bed compared to the apparent uniform distribution of microbial communities throughout the 5 mm sediment, irrespective of depth and location. This is in keeping with the findings of the ex-situ flask experiments assessed in Chapter 3, Section 3.5.3, from which it can be concluded that the lack of infiltration and light penetration through the smaller interstitial pore spaces in the sediment bed are rate limiting factors for microbial development at depth in fine sediments. Subsequently, this explains the thick ‘mat’ of microbial accumulation on the surface of the 0.5 mm sediment, as the concentration of light and nutrients at the surface results in high microbial population density.

Figure 3.17 illustrates the differences between biofilm developments on the surface of the experiments, indicating that the microbial community development is comparable at the surface layer of the sediments irrespective of sediment particle size, with the microbial community levels increasing at a similar rate in the different experimental test systems. This indicated that the inoculum source provided a suitable microbial community to promote biofilm development whilst suggesting that the microbial levels were similar in the water employed in the tests. Figure 3.17 does indicate a higher level of biofilm presence in the 0.5 mm sediment system at the surface, however, this is not significantly different based on ANOVA (P=0.32).

Figure 3.18 enumerates the difference between the microbial community developments at depth in the two differing sediment systems. The figure demonstrates that there is a reduction in microbial community presence from Time 0 to Time End in both experimental tests, inferred from the glucose levels measured from the extracted core sections. This is comparable with the findings from Nogaro et al. (2010). However, the 0.5 mm sediment experimental systems start at a higher levels (135 μg/mL) than the 5 mm systems (95 μg/mL), with levels that decrease rapidly after the surface 2 cm of the experimental bed (falling from 80 μg/mL at 2 cm to 24 μg/mL at 3 cm). Conversely, there is a more gradual reduction with depth in the 5 mm test systems, with the microbial community levels falling steadily with increased depth from surface. This suggests that the poor water movement induced by steady pressure gradients at the bed surface (Elliott and Brooks, 1997a) is more influential in the 5 mm sediment bed, most likely attributable to the larger interstitial porous spaces. The water is capable of reaching the entirety of the sediment bed in the 5 mm sediment system faster than in the 0.5 mm system, causing a larger depth penetration of microbial growth as the nutrients are capable of travelling to greater depths. Conversely, the microbial development concentration at the surface of the 0.5 mm sediment systems could be attributable to the same factor, as the finite room in the interstitial pore space in that sediment had the potential to restrict/confine the depth of nutrients and microbial communities to greater depths, promoting their development at the surface.

3.5.4.2 Impact of Microbial Community on Hyporheic Exchange in Flat Bed System

Figure 3.19 shows an example of the variance recorded in the normalised hyporheic exchange trace tests. Figure 3.20 illustrates the measured hyporheic exchange gradients taken from the normalised trace data of the 5 mm and 0.5 mm experimental systems over the course of the 30 tracer experiment. This figure illustrates the variance between the exchange gradients temporally and due to sediment particle size. ANOVA analysis supported similar studies findings and theories by identifying a significant difference in hyporheic exchange based on sediment particle size; exchange is faster in larger sediment ($P=0.034$). Moreover, analysis of parallelism and repeated measure ANOVA indicated that there was no significant change in hyporheic exchange in the 5 mm sediment over the course of the test, irrespective of the quantity of microbial community presence in the system ($P=0.222$). However, ANOVA and analysis of parallelism indicated significant variance in temporal exchange rates based on the exchange gradients in the 0.5 mm test systems ($P<0.01$), indicating a direct effect of microbial development on the surface of the sediment bed on the rate of hyporheic exchange. From this, it can be identified that microbial development predominates at the surface of small sediment particle beds, clogging the interstitial porous spaces over time, subsequently inhibiting the rate of hyporheic exchange by slowing the rate of molecular diffusion into the sediment bed by affecting the water flow through the development of the biofilm, with clogging probably increasing the residence of infiltrating water within the sediments, as seen in the in-situ study on the Usses River, France, by Nogaro et al. (2010) This demonstrates that modifications of water flow patterns can be attributed to the clogging influence of microbial growth and their associated activities in the sediment layers (Brunke and Gonser, 1997; Nogaro et al., 2010).

3.5.4.3 Microbial Community Development on Different Sediment Particle Sizes in Undulating-Bed Experimental system

To identify whether there was any physiological variation in the development of biofilm on different bed-forms, Figures 3.21, 3.22, 3.23 and 3.24 illustrate the visual differences observed in microbial community development on 5 mm and 0.5 mm sediment particle sizes for undulating dunes with varying wavelengths (λ= 2m and 0.2m). These figures collaboratively illustrate the same visual variation in biofilm development between the two different sediment types as seen in the flat bed system in terms of microbial community variation. They show the microbial community predominating on the surface of the 0.5mm sediment particle size systems, developing as a mat and layering the majority of the sediment surface (Figures 3.22 and 3.24). Conversely, the 5mm sediment test systems showed a more uniform distribution of microbial development, with apparent homogenous covering of each independent particle size throughout the system, irrespective of location, by the end of the tests (Figures 3.21 and 3.23).

Figure 3.26 quantifies the microbial development in the experimental systems, showing the variance between surface level microbial development and microbial development at depth on the two different dune systems with both of the experimental substrates. The figure shows that there is variance in levels of microbial development at the different sections of the dune system. In all of the test systems, the glucose levels on the 5 mm sediment test substrates reduce in a steady manner with depth, gradually reducing from the peak values; this is in keeping with what was found in the flat bed test system. However, the 0.5 mm sediment systems show initial high levels for the surface sediment concentration followed by a sudden drop in the maximum to a low level after the top 2.5 cm at all locations of the flume that were analysed within the 2-dune test system (λ= 2m). Differently, the multiple dune system (λ= 0.2m) shows the similar trend in rapid drop in glucose level at depth, but after 3.5 cm, suggesting that there has been a greater level of depth penetration of the sediment bed. This could be attributable to the greater frequency of dunes resulting in a larger level of flow net connectivity, resulting in the spread of nutrients to a higher

depth that has permitted microbial development deeper within the sediment than seen in similar tests, potentially clogging the interstitial porous spaces to a greater level. Figure 3.25 also shows that there is a lower level of microbial development found in the core taken from the down slope of the system when compared to the other sections, implying a lower level of nutrient influx which could have inhibited microbial growth.

Figure 3.26 shows the variation in measured glucose levels at the surface of the sediment beds in the experimental test systems employing different wavelengths. It is apparent that the microbial developments in the systems develop at comparable rates irrespective of bed-form. However, the glucose levels recorded in the 0.5 mm sediment tests were found to be significantly higher at their maximum plateau levels than in the 5 mm sediment particle size tests irrespective of bed-form ($P=0.027$). This is likely attributable to the inhibition of the effective diffusion into the sediment bed as a result of the dense packing of the sediment causing small interstitial pore spaces, resulting in surface development of biofilm to ensure highest levels of microbial nourishment (Nogaro *et al.,* 2010).

3.5.4.4 Impact of Microbial Community on Hyporheic Exchange in Flat Bed System

Impact of microbial development was quantified by temporal repeat discrete injections into the flume systems. Figures 3.27 and 3.28 illustrate the temporal variance of hyporheic exchange on 0.5 mm and 5 mm sediments on the two different experimental treatments (λ= 2m and λ= 0.2m). Both figures identify the fact that there is a significant temporal reduction in measured exchange gradients (P=0.019 and 0.014 respectively), implying that the microbial development has impeded the rate of hyporheic exchange. As mentioned in the analysis of the flat-bed system, this is likely attributable to sediment clogging of the small interstitial sediment particle sizes by biofilm development. However, both figures also demonstrate a lack of significant variation in exchange gradients due to biofilm development over the course of the experimental series (P = 0.11 and 0.067 respectively). This suggests that the 5 mm sediments used in the test system are too large to permit microbial development on the surface, meaning that the pore spaces will not become clogged. This is seen through the development of biofilm around each independent particle irrespective of depth.

It should be noted that microbial community development is cyclical. This means that at some point in its developmental cycle, parts of the microbial community may detach and survive within the water column as suspended sessile biofilms (see Figure 1.3). There is the possibility that the biofilm community may reduce in the latter stages of the test and not impede exchange. Figure 3.29 shows the analysis of the multiple dune test (λ= 0.2 m) after 18 days, thereby omitting the final day of sampling. In doing so, the ANOVA and analysis of parallelism showed significant temporal reduction for both the 0.05 mm and 5 mm sediments (P=0.014 and P=0.047). This suggests that there is the possibility that microbial community development could impede hyporheic exchange on large sediments, but is dependent upon the biofilm stability. Essentially, if the microbial community develops to the point at which sloughing occurs, any inhibition on exchange that has been caused by sediment clogging may be undone as the microbial cells erode from the biofilm and become floating motile organisms.

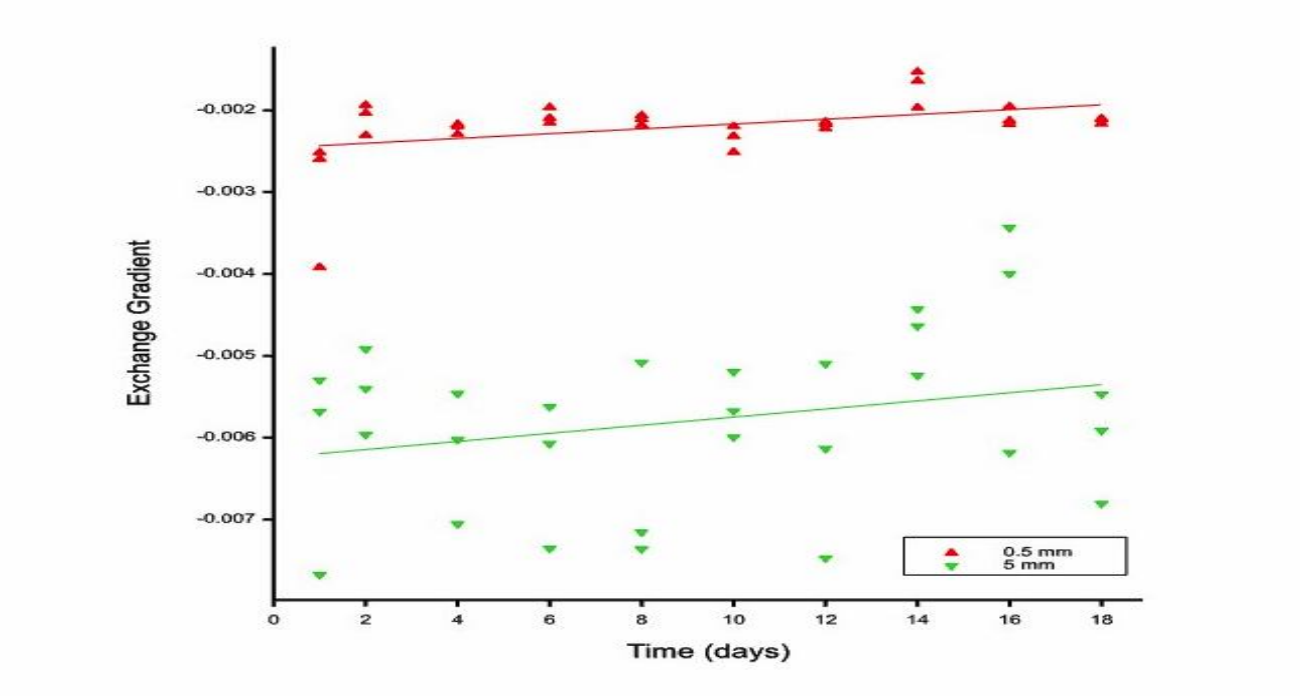


Figure 3.29: Temporal variance in normalised hyporheic exchange gradients from multiple dunes flume tests; omitting last sample

To quantify this, further experimentation is required. The experimental system employed in this study could be modified to incorporate a filter in the weir system that could be removed at 24-hour intervals to measure the microbial community levels transported within the water column over time. If the level of biomass were to increase at different intervals during the course of the test, it could then be compared directly to whether or not there had been a significant reduction in exchange, potentially quantifying the effect of sloughing on interstitial sediment clogging.

Chapter 4

Assessment of the transformation rate of p-nitrophenol in aquatic sediment systems; utilizing inter sample comparison to evaluate spatial variability of chemical degradation rates

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4.1 Introduction

Humanity's expansion and increased use of chemicals resulted in the degradation of lotic ecosystems. Industrialisation, urbanisation and land use change alters aquatic environments through the input of organic waste (Finnegan *et al.*, 2009; Vörösmarty *et al.,* 2010). Such chemical pollutant influx into aquatic systems detrimentally impacts the habitability of the environment, causing shifts in biotic diversity, dominance and functionality (Lake, 2000; Dyer *et al.*, 2003). Models have been created to assess risk and predict exposure distance and duration in the event of chemical entry into river systems (Technical Guidance Document, 2003 and the Impact Zone Model; see Paul *et al.,* 2005). Recognized Organization for Economic Cooperation and Development (OECD) biodegradation test guidelines have been designed to predict the environmental risk associated with chemicals' persistence and potential ecotoxicological impacts on the environment whilst providing information about the pathways of metabolism in the environment (Jaworska *et al.,* 2002; Yuan *et al*., 2003).

These biodegradation tests employ environmental samples as inoculum, in some instances using biofilms to evaluate chemical biodegradation rates (Reuschenbach *et al.,* 2003; Zhang *et al.*, 2012). The protocols employed in these simulation tests do not account for real world environmental variables, are ambiguous regarding inoculum collection and predominately focus on a single variable (Boethling *et al.,* 2003; Reuschenbach *et al*., 2003). As a result

increased realism is required if these tests are to accurately predict chemical fate in the environment.

4.1.1 Current Testing

4.1.1.1 OECD Tests

Chemical industrial expansion has resulted in the employment of novel chemical compounds in everyday products, ranging from household cleaners to pharmaceutical and personal care products (Fenet *et al*., 2003; Vieno *et al*., 2006; Ramirez *et al*., 2009). These chemicals end up entering the aquatic environment following their use within the home, primarily via wastewater, as 'Down The Drain chemicals.' To quantify persistence and subsequent potential hazardous effects to the environment caused by such chemicals, standardized biodegradation tests have been designed and implemented by the OECD. The majority of these tests have been designed to be conducted under standardised conditions, which may not reflect environmental realism.

4.1.1.2 Factors that could enhance the realism of OECD tests outcome

Provision of reliable degradation data for chemical fate prediction has been the predominant aim of biodegradation tests. However, the biodegradation tests currently employed merely generate a portion of the information needed to establish chemical fate in environmental systems. The complexity of achieving simulation tests that adequately reflect the physico-chemical conditions and nature of the microbial populations in the environment are currently beyond scientific reach. Screening tests have been found to underestimate the rate of chemical degradation in environmental systems (Guhl and Steber, 2006).

A plethora of factors could contribute to the variance in chemical persistence under lab and environmental conditions, including pH variability, light/dark cycle, flow rate of water, oxygen concentrations, riverbed form, test chemical

concentrations, inoculum and inoculum source/density (Battersby and Wilson, 1989; Goodhead et al., 2013; Kunkel and Radke, 2008; Lapertot and Pulgarin, 2006; Li *et al.,* 2005; Lima *et al.,* 2005; Rutgers et al., 1998; Thouand et al., 1995, Thouand et al., 2011). Of these variables, two factors have been poorly studied to date, light abundance and origin of sediment material.

4.1.1.2.1 Light

Light is a major concern in biodegradation studies, especially when investigating the biodegradation of chemical substances using respirometric methods. In such instances, availability of light promotes algal growth, which may result in increased dissolved oxygen levels, and reduced CO_2 levels confounding the measurement of chemical degradation. Furthermore, dissolved oxygen acts as an electron acceptor that is required for aerobic biodegradation of chemicals (Xu *et al*., 2005). Additionally, algal growth can result in altered nutrient availability, potentially impacting the biodegradation dynamics of a test chemical. The availability of light also affects the levels of direct and indirect photolysis of chemicals, where direct photolysis is light-dependent chemical degradation and indirect photolysis is caused by light producing free radicals that induce chemical degradation (Pereira *et al.*, 2007).

Several studies have shown that phototrophic microorganisms are able to degrade chemicals under light conditions. For example, Hirooka *et al*. (2006), found that a mixture of photoautotrophic microorganisms successfully removed chemicals in a liquid medium under light conditions. Studies have also shown that algae can biodegrade aromatic chemicals in mixed algal-bacterial culture systems (Borde *et al*., 2003). Studies such as these confirm that algae may be a driving force in chemical biodegradation in surface waters, and the exclusion of light in current OECD test systems may result in the omission of a key biological process responsible for chemical degradation in the environment.

4.1.1.2.2 Spatial variability of degradation rates in aquatic systems

It has been noted that sediment characteristics can affect the rate of chemical biodegradation. This is accounted for within OECD tests, which state that it is necessary to use two differing sediment types, clay and sand, to assess the biodegradation (OECD 306). The calculated mean biodegradation rates of the two different sediments are then used to quantify biodegradation, due to the potential variability in the impact of different sediments on biodegradation rates both within and between sites (OECD 306).

While use of two sediment types provides ease of control within degradation tests in terms of reliability and reproducibility (Reuschenbach *et al.,* 2003), in the natural environment, sediments show considerable variability both within and between compartments, including the depth and properties of water, properties of sediment materials, the nature of bed-forms, particle size, and sediment uniformity (Ericson, 2007; Kunkel and Radke, 2008). These factors may control degradation by affecting pH (Rutger *et al.*, 1998; Singh *et al.*, 2006; Li *et al.*, 2008), temperature (Kang and Kondo, 2002; Singh *et al.*, 2006), light (Li *et al.*, 2005; Roland *et al.*, 1998) and oxygen (Bhatti *et al.*, 2002). Additionally, in natural river systems, the sediment dynamics and channel velocity potentially affects attenuation of chemicals. This determines the chemical interactions with bacteria on the sediment surface (Kunkel and Radke, 2008), thereby making the sediment interactions more important.

Kleka *et al.* (2001) investigated the variability in biodegradation of bisphenol-A (BPA) in river waters from seven differing watercourses across the United States and found that BPA degrading microorganisms are widely distributed in nature. Mineralisation of BPA occurred in all rivers following lag phases ranging from 2-4 days, with short half lives for BPA biodegradation regardless of geographic location, sampling site or the presence of sediment. Conversely, comparative research has demonstrated that origin of river and lake sediments (Pesce *et al.*, 2013; Masuda *et al.*, 2012) greatly impacts the rate of chemical

degradation. These studies demonstrated that the rates of biodegradation were faster in systems using river sediments. However, there is currently limited research assessing variability in the rate of chemical degradation along a single watercourse.

Moreover, the manner in which the sediment is processed for use in laboratory biodegradation tests could affect chemical degradation rates. OECD test guidelines call for the sieving of sediment samples used in testing to < 2 mm. This sieving alters the sediment structure, composition and microbial community structure, potentially altering capacity for biodegradation relative to the natural environment.

Riverine systems vary in terms of their chemical composition, geology, topography, inputs and climate, directly impacting the form and function of microbial communities in streams (Hall *et al.,* 2012). Studies have shown that microbial community composition between biomes varies due to differences in the organic matter entering these systems (Findlay and Sinsabaugh, 2006). Other studies identify that variance in nutrient influx has the capacity to affect microbial community respiration, enzyme activity and composition in the hyporheic zone (Findlay et al., 2003). However, limited research has been conducted on the relationship between microbial community structure and function and chemical degradation.

4.1.1.2.3 Chemical Concentration

OECD test regulations state that the chemical levels to be used are at the level of mg/L, which is unrealistic when compared to the ranges found in the natural environment and at wastewater treatment plants (ng/L to µg/L levels). These unrealistically high concentration tests create problems and inaccuracies with data interpretation and alter the accuracy and realism of the tests when extrapolated to predict impacts in the natural environment (Ahtiainen *et al*., 2003). Chemical biodegradation is directly related to chemical concentration. Biodegradation of chemicals at high concentrations is usually a growth-linked

process, while at low concentrations chemical biodegradation may occur through metabolism, without inducing growth, and therefore may be slower (Wang *et al*., 1984; Rhee *et al*., 2002).

Using unrealistically high levels of test compound, in comparison to those found in the environment, provides a means to identify populations that biodegrade the test compound, such as by culturing or the use of culture independent analyses (Johnson *et al*., 2004). However, employment of high chemical concentration in the test systems does not enable accurate prediction of chemical biodegradation rates or kinetics under environmental conditions, as the experimental system does not reflect the processes that occur in the natural environment. This is because the use of such high concentrations may evoke growth and cause unnatural dominance of microorganisms that may only be a minor aspect of the ecosystem structure. As such, enrichment of these microorganisms may elevate the rate of biodegradation and hence provide pseudo, non-realistic results. This suggests that levels of environmental realism should be considered and included in experimental designs of current testing to permit higher levels of understanding of these processes in environmentally relevant conditions, thereby making predictions and models more accurate.

4.1.1.2.4 Para-Nitrophenol

Chemical selection for experimental employment was crucial, as understanding of degradation kinetics and chemical responses to environmental parameters are essential for accurate monitoring. Presently, there are an abundance on chemicals that are employed in studies that assess environmental realism, including *para-nitrophenol* (PNP).

There have been many reports on the biodegradation of PNP (Roldán et al., 1998; Lima et al., 2003; Kowalczyk, 2013). This is because PNP biodegradation pathways, including genes encoding key enzymes involved in its biodegradation, are well characterized (Kitagawa et al., 2004; Perry and Zylstra, 2007; Zhang et al, 2009). Subsequently, 2 major degradation pathways of PNP have been categorized, both of which result in degradation of PNP to

maleylacetate (Figure 4a). PNP biodegradation via hydroquinone has been found in Gram-negative bacteria (Prakash et al., 1996; Spain and Gibson, 1991), whereas PNP biodegradation via 4-nitrocatechol has been found in Gram-positive bacteria (Kadiyala and Spain, 1998). As such, PNP was chosen for employment in this study.

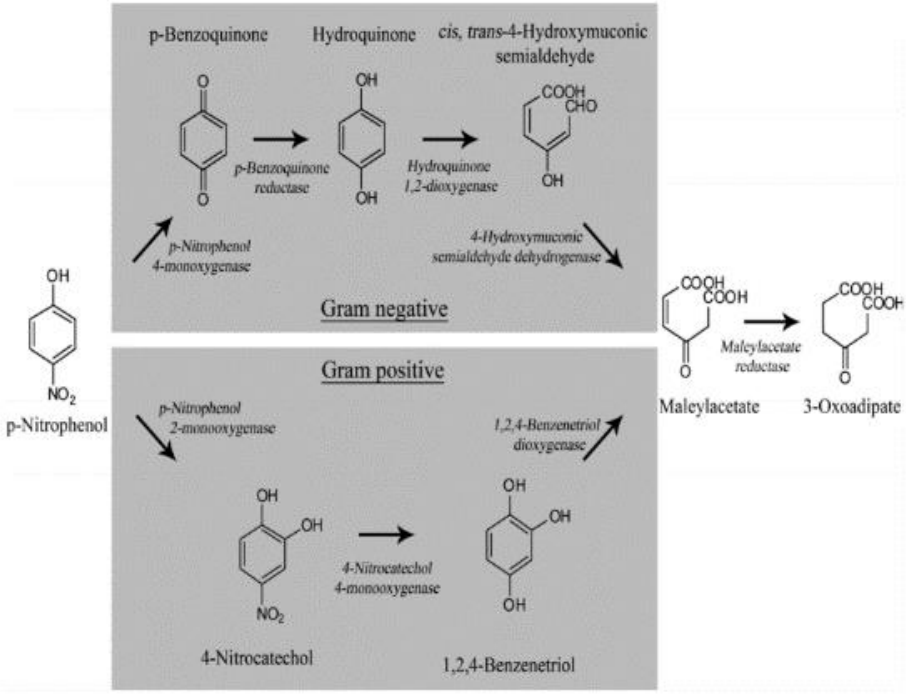


Figure 4a: Pathways for PNP degradation in bacteria
(Source: Chauhan et al., 2010)

4.2 Objectives

The aim of this study was to investigate the impact of sediment presence, sampling site location and sediment composition on the reproducibility of *para*-nitrophenol (PNP) biodegradation tests. This work had the following objectives:

1) Identify the relative capacity of river sediment and water to degrade PNP
2) Determine the impact of light and dark conditions on the rate of PNP degradation
3) Quantify the relative roles of different river sediments and water for PNP degradation in aquatic systems

4.3 Methods

4.3.1 Identify the relative roles of sediment presence and water for PNP degradation in aquatic systems

The biodegradability of *para*-nitrophenol (*PNP)* was assessed in *ex situ* aquatic systems using river sediment from a single watercourse, the River Dene, collected 500 metres downstream of the major Waste Water Treatment Work at Wellesbourne. The experimental protocol of OECD test 308 was followed and the test was conducted in the dark to remove light as a variable. Experimental units were set up to compare chemical biodegradation rates of river water and sediment in water systems, permitting assessment of chemical biodegradation using HPLC.

4.3.1.1 Sample Collection

In September 2013, water samples were obtained from the River Dene 540 m downstream of the Wellesbourne Wastewater Treatment Plant (WWTP) effluent discharge point (52˚11’58.54’’N and 1˚36’44.94’’W). The river water collected was filtered through a 38 µm sieve in order to remove particulate matter and large protozoa. Surface sediment was then collected from the same sample location. Surface river sediment samples were collected using a scoop of surface riverbed sediment between 0 – 10 cm depth (in-keeping with Masuda *et al.,* 2012) as no standard collection techniques or tools are employed for riverbed sediment collection. However, OECD test 106 specifies employing a reproducible technique is beneficial. This sediment was sieved to < 2 mm sediment particle size using a series of sieves and used in the experimental systems to identify the impact of sediment presence on biodegradation. Collected sediments were kept moist and, prior to experimentation, moisture content of sediment was calculated to identify the weight of sample needed for each experimental unit; 50g dry weight sediment was required in each experimental unit (see Avnimelech *et al.*, 2001).

The river water and sediment were then used as inoculum in PNP biodegradation experiments. River water for the controls was sterilized in three autoclaving cycles (121 ˚C, for 15 minutes, 1.1 atm). Biodegradation tests were carried out according to guideline OECD 308.

4.3.1.2 Sample Collection Site

The water used for this test series was collected in the same manner as that described in Section 3.3.1.2. The surface sediment was collected as described in Section 4.3.1.1 using a metal shovel, which was used to penetrate the top 5 cm of the sediment bed for the sample collection. The sample site is illustrated in Figure 4.1.

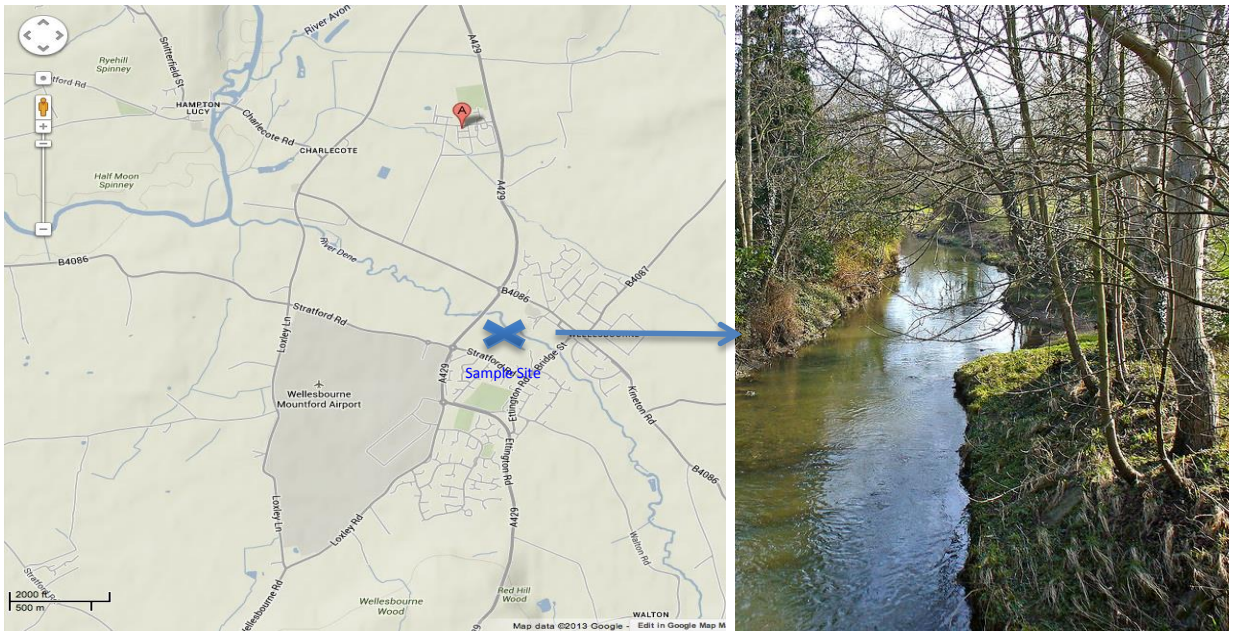


Figure 4.1: Study site for sediment and water collection along the River Dene, Wellesbourne, UK.

4.3.1.3 Experimental set up

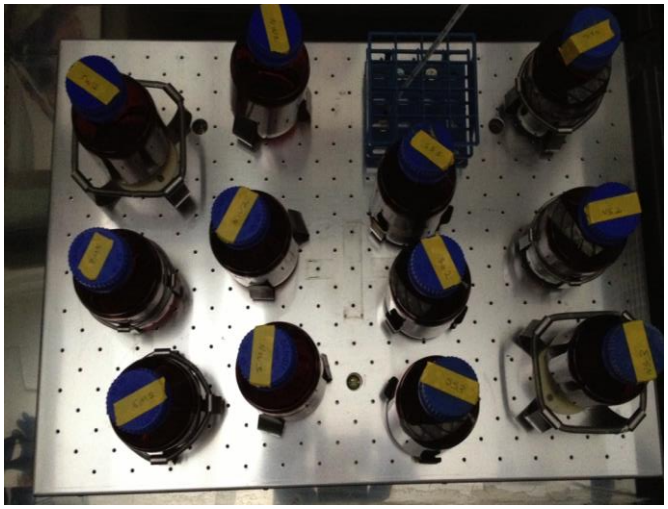
500 mL sterile amber Duran Schott bottles (Fisher Scientific, UK) were used to simulate dark conditions. The amber bottles inhibited light penetration into the experimental units. This investigation employed 4 different experimental treatments:

1) Sterile Water
2) River Water
3) River Sediment and Sterile Water
4) River Sediment and River Water

The bottles were filled with their respective treatments, which acted as inoculum. The sediment was sieved to ≤ 2 mm and the river water filtered through a 38 µm mesh, in keeping with similar experimental studies and OECD test regulations (Ericson, 2007; Masuda *et al.,* 2012; Zhang *et al.*, 2012). Following this, 50g dry weight equivalent sediment was added to the Duran bottles that required sediment as part of the experimental treatment (treatment 3 and 4). 150 mL of filtered river water was then added to the systems, maintaining a 3:1 (water: sediment) ratio (Ericson, 2007). The experimental units that employed solely water were filled with 200 mL of river water or sterilized river water respectively. The samples were then placed onto rotary shakers (MK V Shaker, L.A. Engineering Co., UK) at 20 ˚C and 50 RPM to pre-incubate for 7 days. Treatments and controls were prepared in triplicate.

After the 7-day pre-incubation period the water was removed from each vessel and replaced with fresh river water. The replacement river water was dosed with a concentration of 2 mg/l of PNP; a 3:1 water: sediment ratio was maintained. The Duran bottles were then replaced onto the rotary shakers in a randomized order at 50 RPM, at 20 ˚C. At 24-hour intervals 1 mL aliquots of water were removed from each bottle in a fume hood and concentrations of PNP were quantified by high-performance liquid chromatography (HPLC) until complete degradation was observed.

The experiment (Figure 4.2) was conducted in a controlled environment room. The rotary shaker used maintained a constant incubation temperature at 20˚C ± 2 ˚C, and bottles were incubated under constant motion using the rotary shaker.



Experimental Units:

- 3:1 water: sediment ratio
- 7 day pre-incubation period followed by replacement of water with freshly collected river water dosed to 2mg/l with *PNP.*
- Daily 1ml aliquots taken for HPLC.
- Duran bottles on a rotary shaker at 50 RPM and 20^{o}C
- Amber Duran bottles used for dark experiment to prevent light penetration

Figure 4.2: Experimental units on rotary shaker, incubated in dark conditions with PNP

4.3.1.4 Measurement of PNP biodegradation

At 24-hour intervals, 1 mL aliquots of water were removed from each experimental unit and PNP levels were quantified using High Performance Liquid Chromatography (HPLC). The analysis was conducted on a Hewlett Packard 1100 HPLC machine using a LiChrosphere (5 µm) C-18 column (MerckMillipore, UK). The mobile phase was a mixture of water: methanol (HPLC grade, Fisher Scientific, UK) with a ratio of 40:60 and the flow rate was 0.50 mL/minute. Analysis was undertaken with UV detection at 254 nm, with column pressure of 115 bar and column temperature at 25 ˚C. The volume of the injected sample was 20 µL and the run time was 13 minutes. Standard solutions were prepared containing PNP. HPLC calibration was carried out using concentrations ranging from 2 mg/L to 2 µg/L for PNP. The limit for the lower level of detection for PNP was 20 µg/L.

4.3.2 Determine the impact of light and dark conditions on the rate of PNP degradation

The biodegradability of *para*-nitrophenol (*PNP)* was assessed in *ex situ* aquatic systems using river sediment from a single watercourse, the River Dene. To further the understanding of the impact that sediment presence has on chemical biodegradation, light and dark conditions were employed in this study to identify its impact on the rate of biodegradation.

4.3.2.1 Sample Collection

In October 2013, samples were obtained from the River Dene 540 m downstream of the Wellesbourne Wastewater Treatment Plant (WWTP) effluent discharge point. The sediment and water used for this test series was collected in the same manner as that described in Section 4.3.1.1.

4.3.2.2 Sample Collection Site

The sediment and water used was collected from the study site described in Section 4.3.1.2.

4.3.2.3 Experimental set up

500 mL sterile Duran Schott bottles (Fisher Scientific, UK) were used. Amber Duran bottles were used to stimulate dark conditions and clear Duran bottles were used for treatments exposed to light conditions. The amber bottles inhibited light penetration into the experimental units. This investigation employed 2 different experimental treatments in the two differing light conditions:

1a. River Water - Light
1b. River Water - Dark
2a. River Sediment and River Water - Light
2b. River Sediment and River Water – Dark

The experimental procedure and methodology were consistent with those described in Section 4.3.1.3. However, due to the added light parameter investigated, lighting requirements were integrated into the experimental design.

The experiment was conducted in a controlled environment room with an 18-hour light (white light source with intensity of 7450 lux) and six hour dark cycle. The incubation temperature was maintained at 20˚C ± 2˚C, and bottles were incubated under constant motion using the rotary shaker at 50 rpm. This is illustrated in Figure 4.3. PNP dosing and sampling was conducted as described previously in sections 4.3.1.3 and 4.3.14.

Figure 4.3: Experimental units on rotary shaker in controlled environment room, incubated in light and dark conditions with PNP

4.3.2.4 Measurement of PNP biodegradation

Quantification of PNP degradation was recorded at 24-hour intervals. 1 mL aliquots of water were removed from each experimental unit and PNP levels were measured using HPLC, as described in Section 4.3.1.4.

4.3.2.5 Measurement of Chlorophyll-a

Pigments were extracted from phytoplankton with 90% acetone for 18 to 24 h in the dark on a rotary shaker. The extract's optical density was measured spectrophotometrically. Chlorophyll-a concentrations (pg chl a 1-l) were calculated using the formula from Strickland & Parsons (1972).

Chlorophyll-a was measured by filtering a known amount of sample water through a glass fiber filter. The filter paper itself was used for the analysis. The filter was ground up in an acetone solution and a spectrophotometer was used to read the light transmission at a given wavelength, which in turn was used to calculate the concentration of chlorophyll a.

4.3.3 Identify the relative roles of different river sediments and water for PNP degradation in aquatic systems

To further investigate the accuracy of chemical biodegradability tests, the biodegradability of PNP was assessed in *ex situ* aquatic systems using sediments from different sample locations along a single watercourse, the River Dene. The selected watercourse has eight Waste Water Treatment Plants (WWTPs) within its catchment, a variety of different sediment sections and countless fluctuations in flow.

This experiment primarily established whether variance in sediment properties impact the rate of chemical degradation as OECD 308 specifies that sediment impact on chemical degradation should be determined by averaging the impact of a fine silt and a thick clay on degradation tests.

In this experiment, river sediment was collected from sample sites that possess different sediment characteristics along the length of the main river and its tributaries, permitting comparison of sediment. There were 6 sample sites, each with different sediment properties; 3 samples were collected across a transect along the riverbed at each site. The OECD 308 guidelines were followed and the sediments were sieved to a 2 mm particle size and placed in amber durans (to remove light as a variable) with river water on a rotary shaker at 50 RPM and 20˚C. There was also a treatment of unsieved sediment from a 7th sample site and a control of just river water.

4.3.3.1 Sample Collection

In November 2013, samples were obtained from the River Dene, at different locations along the watercourse. The sediment and water used for this test series was collected in the same manner as that described in Section 4.3.1.1.

4.3.3.2 Sample Collection Site

The River Dene is a minor tributary of the River Avon, Warwickshire UK; it flows approximately 16 km from its origin in Burton Dasset Hills in Kineton to its confluence near Charlecote Park. There is a mixture of land use, such as nearby urban transport routes, residential and commercial areas, and predominant agricultural use, surrounding the watercourse. The river has previously been used as a location of inoculum for chemical biodegradation tests (Singh *et al.*, 2006; Kowalczyk, 2013) attributable to its identification as a "good' to 'very good' biologically diverse unpolluted river (EA, 2009).

The River Dene has five Wastewater Treatment Plants (WWTPs) along its watercourse and three WWTPs on tributary streams that flow into it, illustrated in Figure 4.4. The contrasting size, influxes and treatment strategies employed at each WWTP results in varying chemical and nutrient influxes into the water course, potentially generating variance in the biological community within the river sediments.

Sampling was conducted in the River Dene catchment area, providing a total of 6 manually selected sample locations with different sediment properties, illustrated in Figure 4.4. Accessible sample site locations were identified in advance of sampling. At each sampling site, a river sediment sample was taken at three equidistant points along transects across the river channel to permit inter sample comparison between sites and sediment properties. The different biological and physiochemical attributes of each sampling site were logged to permit comparison of the sediment samples (sediment type and particle size, velocity, flow depth, pH, water temperature and chlorophyll biomass levels via PLFA).

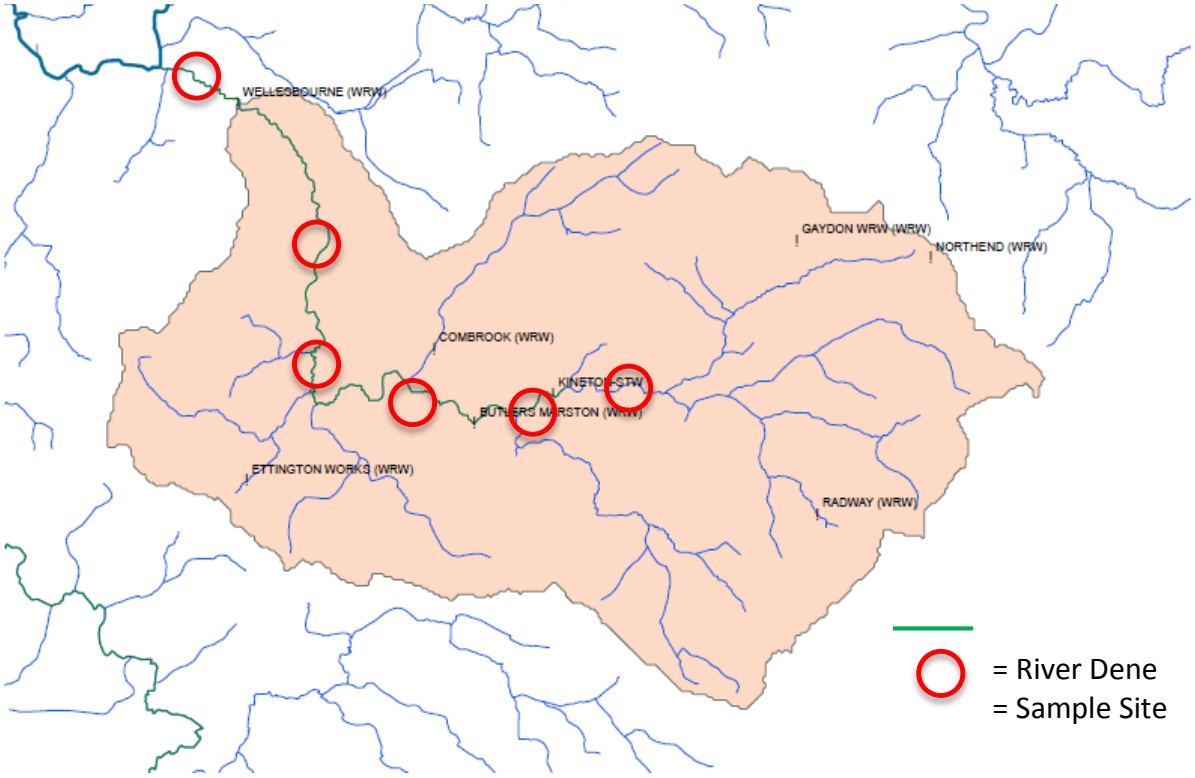


Figure 4.4: River Dene catchment showing distribution of Wastewater Treatment Plants along the watercourse in conjunction with the sample sites.
(Source: Williams, R., Centre for Ecology and Hydrology, UK)

Surface river sediment samples were collected from each location, as described in Section 4.3.1.2. Once collected, the sediment samples were placed into pre-labeled containers and transported back to the lab for sorting. River water was collected from one point source location to be used for all experimental units following sterilization. Sterile and non-sterile river water will be used as controls.

4.3.3.3 Experimental set up

500 mL sterile amber Duran Schott bottles (Fisher Scientific, UK) were used. This investigation employed 5 different experimental treatment sites, with two samples repeated to permit comparison of sieved and un-sieved sediments. The sieving, which is a standardised practice in OECD test methodology, has the capacity to impact the microbial community abundance and diversity within a sample due to the physical disruption that it undergoes. As such, this investigation into its impact is necessary.

The experimental procedure and methodology followed those described in Section 4.3.1.3. However, due to the added un-sieved parameter, two samples were not sieved. Moreover, at the start and end of the tests, the biomass and structure of microbial communities in each treatment was determined using phospholipid fatty acid analysis (PLFA). Briefly, 50 g of soil was freeze-dried and then ball milled (<250 µm) prior to extracting PLFAs. PLFA analysis was conducted at the Macaulay Land Use Research Institute, as described by Thornton *et al.*, 2011. Identification of the lipid fractions was performed by evaluation of standard qualitative bacterial and fungal lipids using acid ester mix reagents, covering a range of lipids between C11 and C20 (Grayston et al. 2001). The quantification of PLFAs was performed by GC chromatography (GC Agilent 6890- Agilent Technology, Santa Clara-USA) using a flame ionisation detector, split injector and HP7673 auto sampler. A capillary gas liquid chromatography column (50 m × 200 µm × 0.33 µm film thickness, coated with 0.5 % phenyl methyl siloxane) was used. Frostegard and Baath (1996) and Zogg et al. (1997) were used for fatty acid nomenclature and to differentiate specific PLFA markers as indicators of fungal, Gram-positive, Gram-negative and actinobacterial markers. The soil sediment analysis was conducted at the Forest Research Centre for Ecosystems, Society and Biosecurity, in keeping with the ISO 11277 method for Particle Size Analysis (Loveland and Whalley, 2000).

4.3.3.4 Measurement of PNP biodegradation

Quantification of PNP degradation was recorded at 24-hour intervals. 1 mL aliquots of water were removed from each experimental unit and PNP levels were measured using HPLC, as described in Section 4.3.1.4.

4.3.4 Statistical analyses

4.3.4.1 Analysis of HPLC degradation rates

Analysis of variance (ANOVA) was used to identify the significance of differences ($P<0.05$) in PNP biodegradation and the associated microbial communities between treatments. Based on the HPLC measurements for PNP biodegradation time to 90% PNP-degradation (DT_{90}), the length of the lag phase and maximum PNP biodegradation rates were calculated using the model of best fit to the biodegradation kinetics which was determined for each sample, as described by Rodriguez-Cruz *et al*. (2006). All statistical analyses were performed using GenStat (13th edition, VSN International Ltd.).

4.3.4.2 Statistical analysis of PLFA and soil sediment composition

Abundance of all PLFA was pooled to give total abundance per sample. Analysis of variance (ANOVA) was used to identify the significance of differences ($P<0.05$) in total PLFA and soil sediment composition levels between sample sites. Subsequently, MANOVA and Tukey Post Hoc tests were conducted to identify whether any of the sediment particle sizes or PLFA compositions significantly differed from each other. Visualization of these significant differences was conducted using Canonical Variate analysis.

4.4 Results

4.4.1 Impact of river sediment and water for PNP degradation in aquatic systems

Complete degradation of PNP occurred after 6 days in all treatments employing sediment, irrespective of the water used in the treatment (Fig 4.5). Degradation in sediment systems was significantly faster than the biodegradation rates of PNP observed in treatments containing only non-sterile river water ($p < 0.05$), which took over 12 days to completely degrade. This can be seen in Table 4.1, which illustrates the significant difference in biodegradation rates between treatments that involved sediment and those that only had river water. Conversely, the treatment containing sterile water underwent no degradation of PNP over the duration of the test.

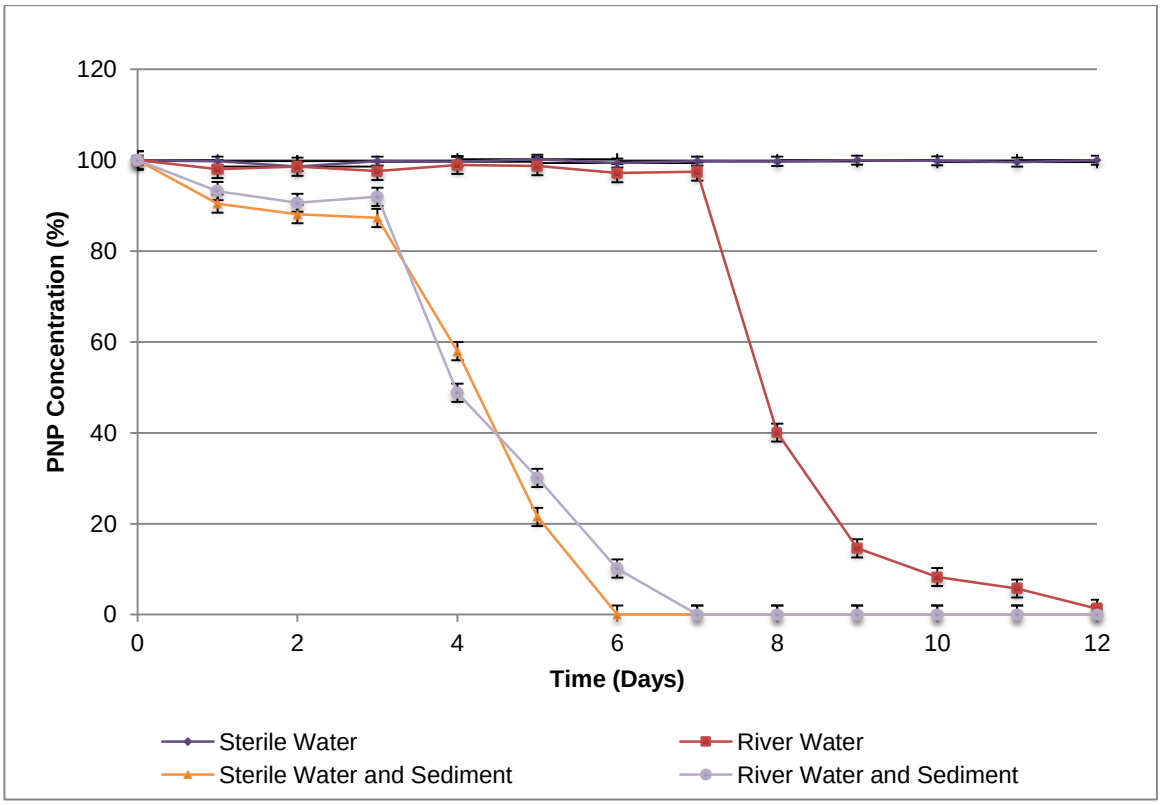


Figure 4.5: The mean biodegradation of PNP under employment of different physiological components. Error bars represent ± standard error of the mean.

Table 4.1: Mean biodegradation rates of PNP recorded from experimental test units with differing physiological components (showing standard error)

Sample	DT15	DT50	DT90
River Water	7.3[a] (± 0.157)	7.9 [a] (± 0.153)	9.6 [a] (± 0.503)
Sediment and River Water	3.2 [b] (± 0.101)	3.95 [b] (± 0.139)	6 [b] (± 0.246)
Sediment and Sterile Water	3.1 [b] (± 0.069)	4.4 [b] (± 0.088)	5.5 [b] (± 0.083)

* Treatments with different letters are significantly different (P<0.05)

4.4.2 Impact of light and dark conditions on the rate of PNP degradation

Degradation only occurred in the systems that were incubated in the dark (Fig 4.6). As seen in Section 4.4.1, sediment and water treatments degraded the PNP faster than the treatments that just utilized water. This is shown in Table 4.2, which highlights the significant difference between the mean time taken for 50% of complete degradation to occur (DT50 values), demonstrating sediment and river water treatments degraded PNP twice as fast as the river water dark treatments, which has been identified as significant by ANOVA analysis ($P < 0.05$). Additionally, Figure 4.6 also illustrates a lack of degradation in all treatments when exposed to light conditions, shown by the lack of degradation within the light experimental units.

Further analysis of the treatments showed significant difference between the pH of systems incubated in the dark compared to those incubated in the light ($P <0.05$). The experimental treatments incubated in the dark showed negligible change in pH over the course of the test, staying at pH levels circa 7.25 (Figure 4.7). Conversely, there was an increase in pH over the duration of the test for treatments exposed to light conditions. This was seen in both the river water and the river water and sediment experimental units that were incubated under light conditions, with the pH rising over the course of the test from 7.25 to above 9.10 over the 20-day test duration.

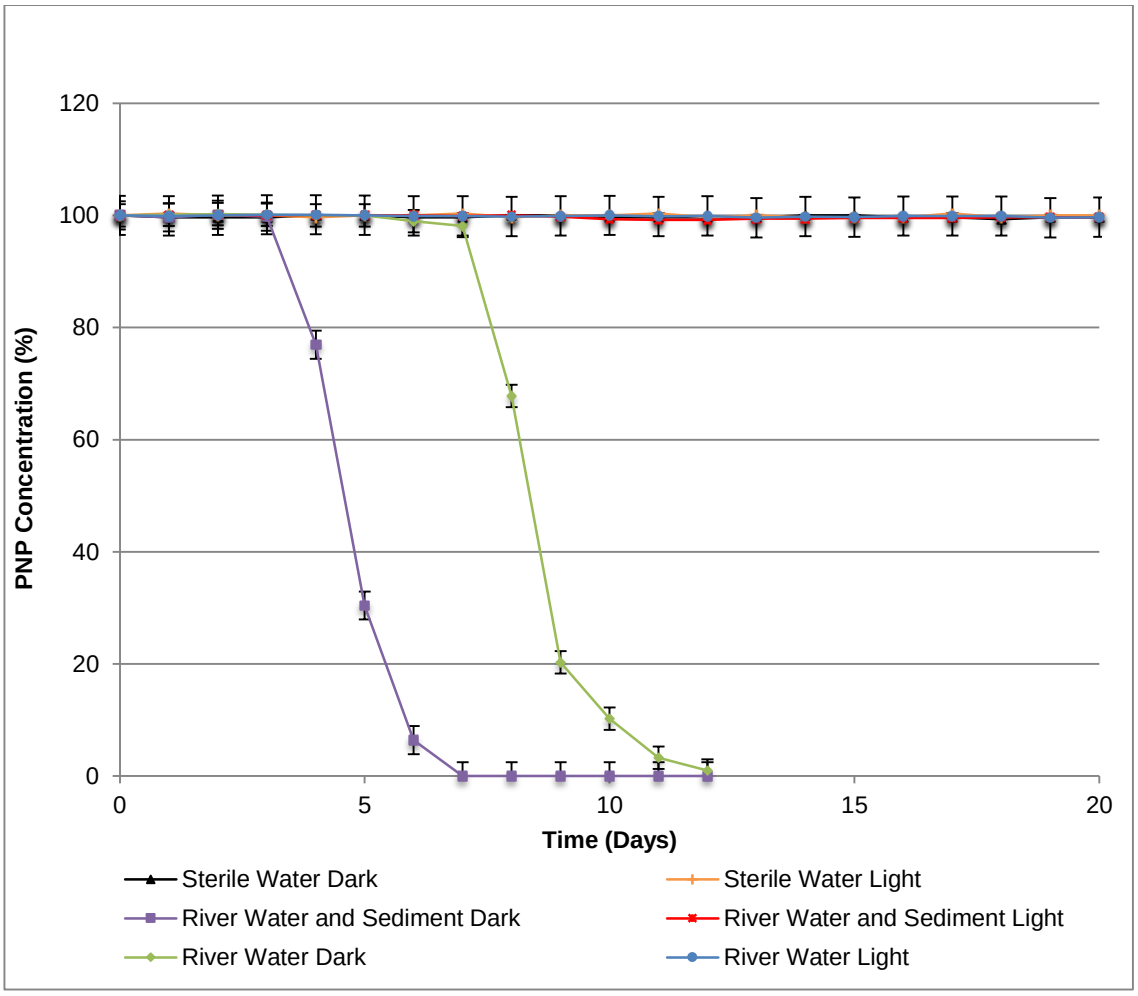


Figure 4.6: The biodegradation of PNP under employment of different light conditions. Error bars represent ± standard error of the mean.

Table 4.2: Mean biodegradation rates of PNP recorded from experimental test units under different light conditions

Sample	DT50	DT90
River Water Dark	8.45 [a] (± 0.142)	10 [a] (± 0.135)
Sediment and River Water Dark	4.7 [b] (± 0.107)	5.8 [b] (± 0.069)
River Water Light	No degradation	No degradation
Sediment and River Water Light	No degradation	No degradation

* Treatments with different letters are significantly different (P<0.05)

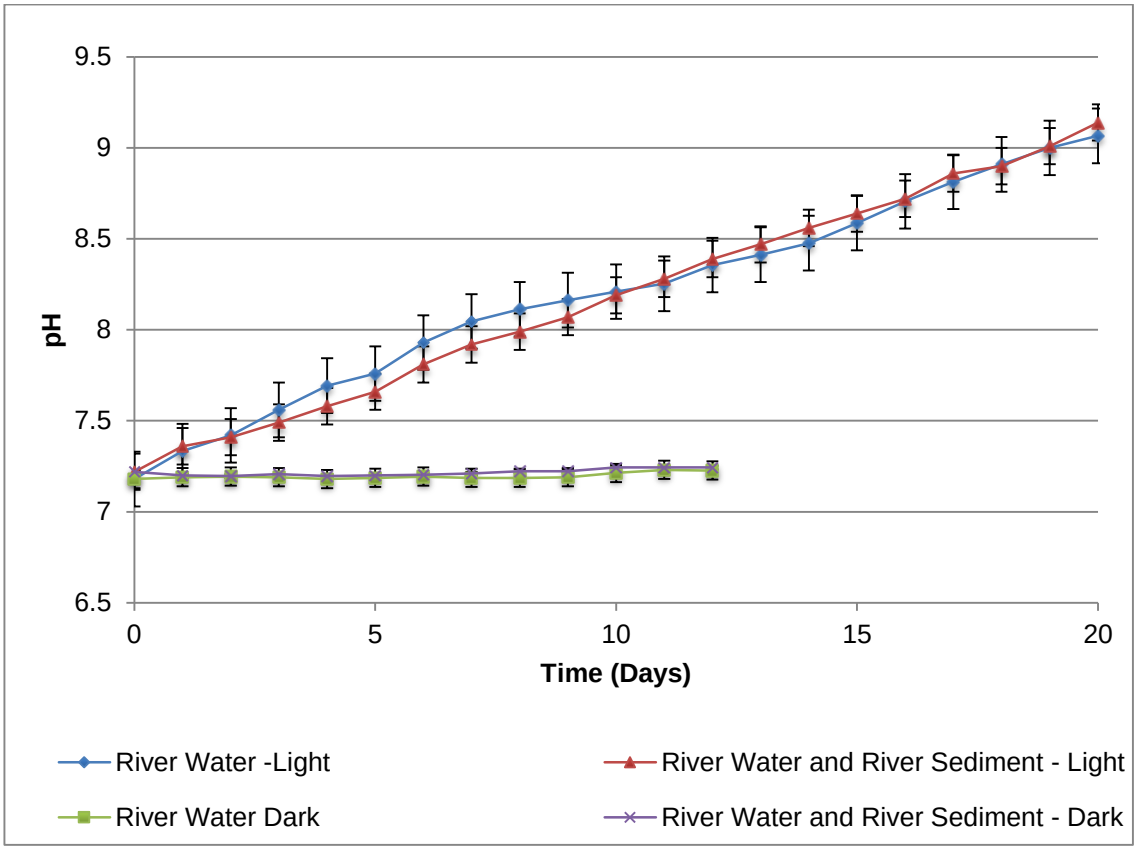


Figure 4.7: Mean pH variation in experimental units during testing under employment of different light conditions. Error bars represent ± standard error of the mean.

A significant difference (P<0.05) was also found in the amount of chlorophyll-a in the light and dark experimental treatments, with higher levels of chlorophyll-a in the light systems, particularly at Time^{End}. This is shown in Figure 4.8, illustrating the significant increase in chlorophyll-a from Time 0 to Time End in the treatments incubated under light conditions, with the chlorophyll-a levels rising from 0.5 to 2.5 in the river water treatments and from 1.5 to 3.5 in the river water and sediment treatments, compared to the negligible increases seen in the treatments incubated in the dark. Moreover, Figure 4.9 shows Canonical Variate Analysis (CVA) using the PLFAs analysed to investigate the effect of treatment and time on microbial community structure. It was found that the soil microbial community structure was significantly different (P<0.05) at the sampling points for Time End Light and Time End Dark samples with most variance on the CV1 axis. This is seen by the lack of overlap of the two Time End treatments within the CVA, indicative of a significant difference with a 95% confidence level.

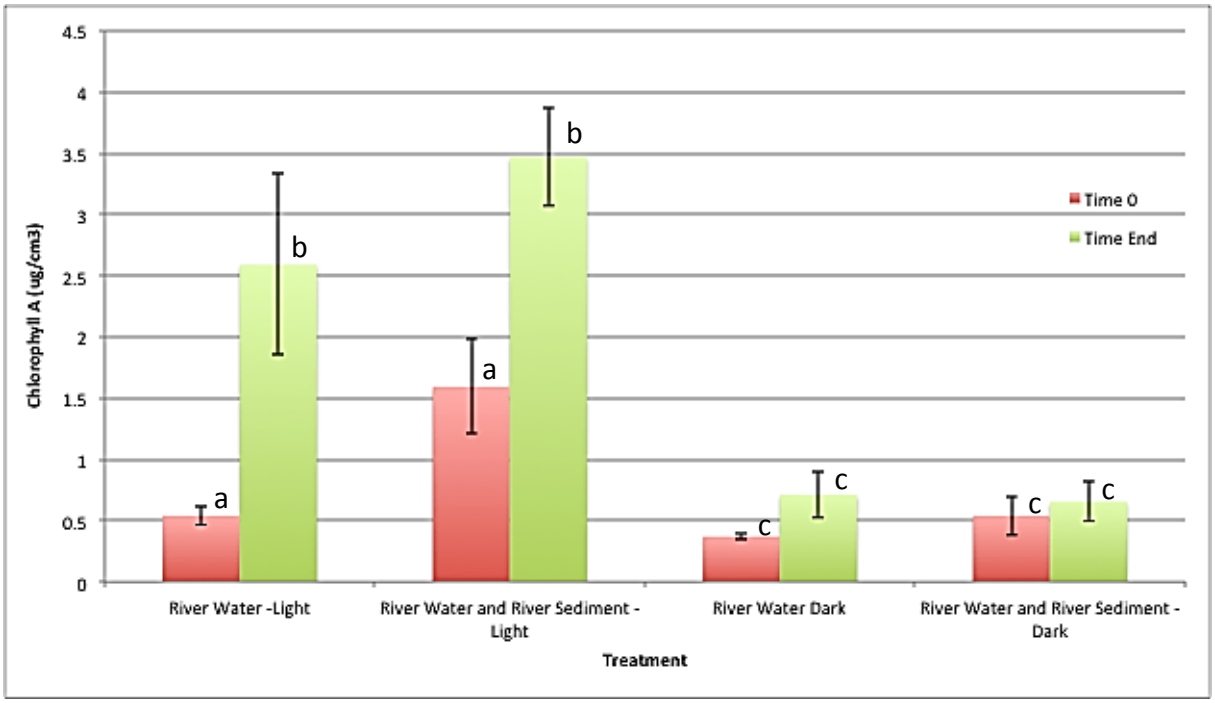
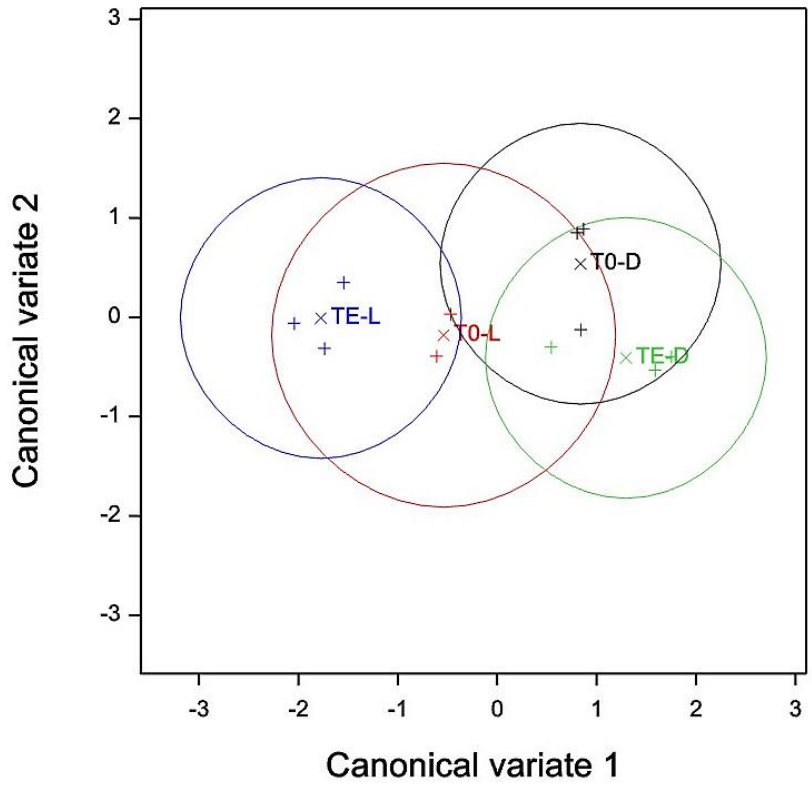


Figure 4.8: Mean chlorophyll-a variation in experimental units during testing under employment of different light conditions. Error bars represent ± standard error of the mean.

* Treatments with different letters are significantly different (P<0.05)



TE-L = Time End Light samples
T0-L = Time 0 Light samples
TE-D = Time End Dark samples
TE-D = Time End Dark samples

Figure 4.9: Canonical variate analysis of Time 0 and TimeEnd PLFA scores from sediment samples.

The canonical analysis assesses the relationship between all of the PLFA quantities for each fatty acid, demonstrating the variance in species abundance in each sample. Circles represent 95 % confidence intervals, with a lap of overlap indicating significant difference between treatment groups.

4.4.3 Variance in different river sediments and water for PNP degradation in aquatic systems

Figure 4.10 shows the variance recorded in the biodegradation rates of varying sediment types collected from the River Dene. Chemical biodegradation of PNP was faster in sites 1, 2 and 3 (irrespective of whether the samples were sieved or not) as opposed to sites 4 and 5, with DT50 being reached before 6.5 days compared to sites 4, 5 and 4 un-sieved, which surpassed this (7.5 days, 8 days and 9.9 days respectively). Figure 4.10 also shows variance in lag time of PNP biodegradation, with Site 1 unsieved and site 5 treatments degrading PNP fastest. Sites 4 and 5 samples were found to have a significantly slower lag phase, resulting in DT50 values surpassing 8 days ($P<0.05$). Additionally, Site 4 (unsieved) displayed a significantly slower rate of PNP degradation when compared to all the other treatments ($P<0.0001$). Quantification of difference in degradation rates was done using two-way ANOVAs with a post hoc Tukey test showing significant differences in degradation rates based on sediment origin employment, with site 4 and 5 significantly slower at degrading PNP ($p<0.001$). The analysis showed a significant treatment effect and a significant change over time ($p<0.001$), with all the treatments degrading *PNP*. The post hoc Tukey test identified Site 4 (unsieved) as degrading at a significantly slower rate than all the other treatments (DT50 = 10 days). The unsieved sample was also found to possess significantly higher levels of chlorophyll-a within the sediment, with 7.8 $\mu g/cm^3$ compared to 3 $\mu g/cm^3$ in its sieved counterpart (Figure 4.11).

To assess the microbial composition of the sediment, PLFA analysis was conducted to see if there was any site related variability in the microbial community, quantifying the association of biomass levels. This data was analysed using Canonical variate analysis (CVA) to identify the PLFA profiles in different river sediment sample sites following extraction from the river (Figure 4.12). The CVA shows a significant difference between Site e (unsieved clay) and all other sediments, illustrated with a 95% confidence interval circle. All the other treatments consist of comparable PLFA profiles, hence the lack of

significant differences between their PLFA profiles (Figure 4.12). Table 4.3 compares the physiological composition of the sediment from the different sites with the DT5 values and PLFA content. This table supports the findings of the CVA, illustrating that site 4 unsieved is significantly different to all the other sample sites, with post hoc Tukey tests showing 95 % confidence ($P<0.0001$). Additionally, Table 4.3 shows that the organic content in the unsieved samples is significantly higher than all of the sieved samples, as shown by site 1 unsieved possessing 2.32 % organic matter and site 4 unsieved possessing 2.30 % organic matter. Moreover, Table 4.3 also shows the site 4 unsieved sample as possessing significantly different sediment composition (68.33 % sand and 21 % clay) and biomass presence (1.69 µg/g) when compared to the other samples ($P<0.05$).

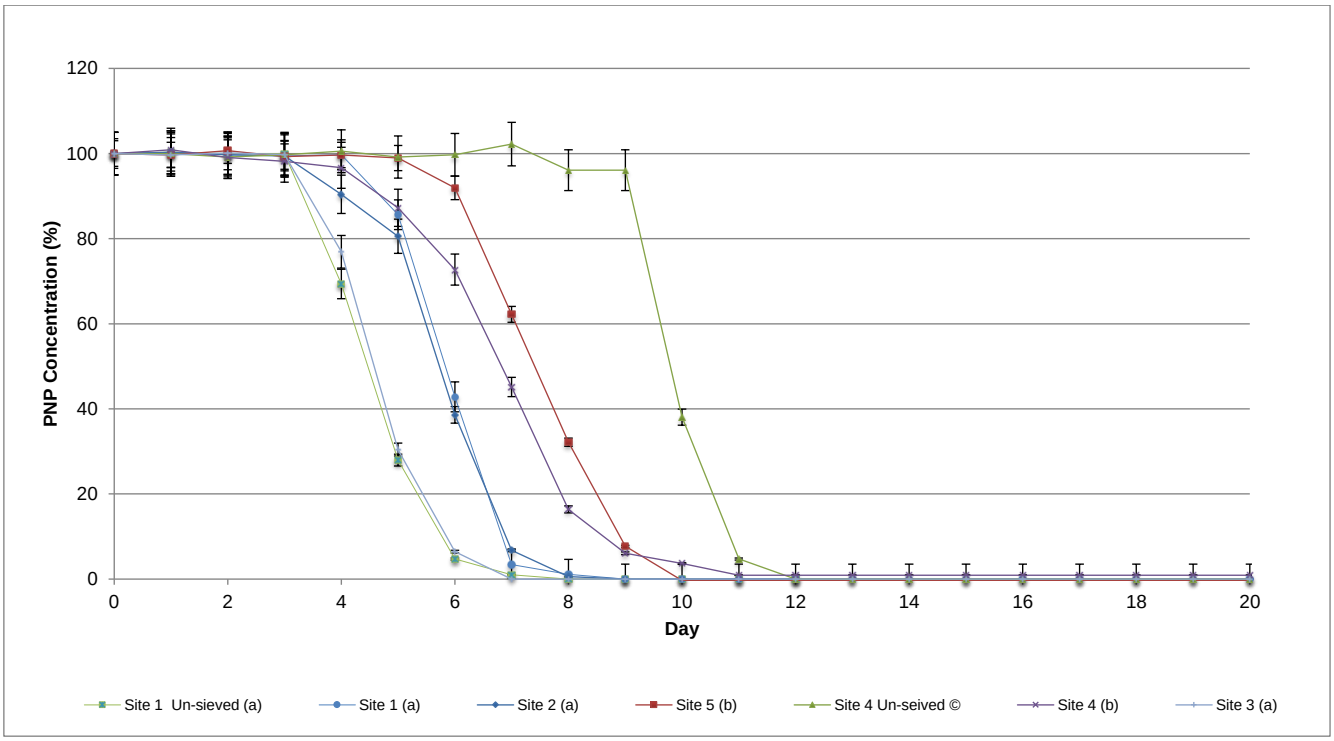


Figure 4.10: The biodegradation of PNP under employment of different sediment types taken from the River Dene. Error bars represent ± standard error of the mean. * Treatments with different letters are significantly different (P<0.05)

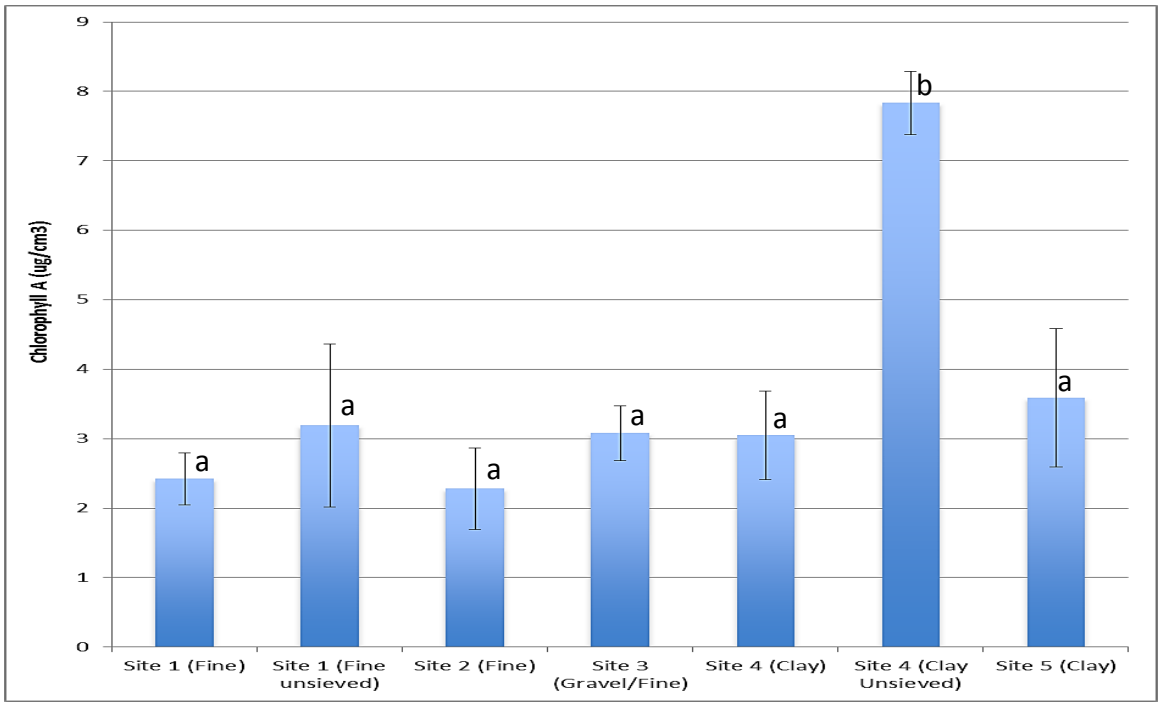


Figure 4.11: Chlorophyll-a content of sediment from different sample sites along the River Dene. Error bars represent ± standard error of the mean.

* Treatments with different letters are significantly different (P<0.05)

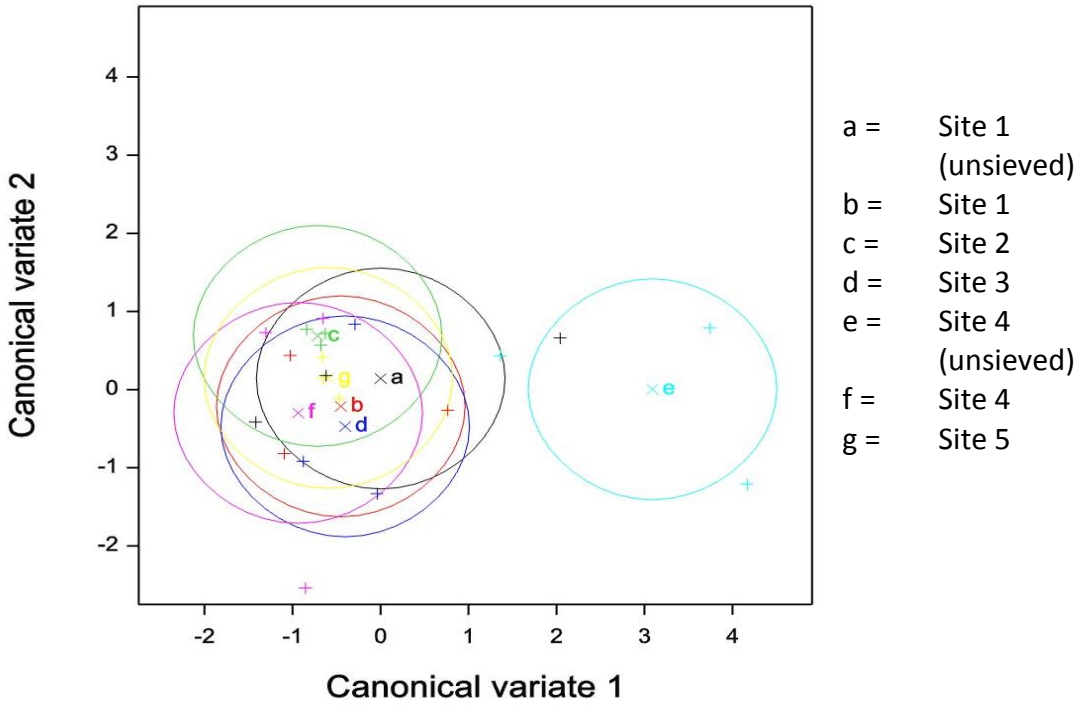


Figure 4.12: Canonical variate analysis plot of PLFA profiles in different river sediment sample sites following extraction from the river.

Canonical analysis assesses the relationship between all of the PLFA quantities for each fatty acid, demonstrating the variance in species abundance in each sample. Circles represent 95 % confidence intervals, with a lap of overlap indicating significant difference between treatment groups.

Table 4.3: Sample site characteristic analysis showing proportion of sediment composition, organic content and total biomass compared against DT50

Sample Site	Clay (0-2µm) (%)	Silt (2-63µm) (%)	Sand (63µm-2mm) (%)	Organic Matter (%)	Total biomass from PLFA (µg/g)	DT50
Site 1	11.33 [a] (±1.02)	8.00 [a] (±0.67)	80.67 [a] (±1.68)	1.98 [a] (±0.040)	1.23 [a] (±0.12)	5.9 [a] (±0.33)
Site 1 (Un-sieved)	6.67 [a] (±3.08)	6.00 [a] (±0.33)	87.33 [a] (±0.38)	2.32 [b] (±0.72)	1.49 [a] (±0.16)	4.4 [a] (±0.107)
Site 2	7.33 [a] (±0.51)	8.00 [a] (±0.58)	84.67 [a] (±1.02)	1.87 [a] (±0.027)	0.79 [b] (±0.03)	5.8 [a] (±0.83)
Site 3	6.33 [a] (±0.84)	8.00 [a] (±0.67)	85.67 [a] (±1.50)	1.92 [a] (±0.039)	1.31 [a] (±0.30)	4.5 [a] (±0.05)
Site 4	9.00 [a] (±0.33)	12.33 [a] (±1.07)	78.67 [a] (±0.77)	1.29 [a] (±0.024)	1.43 [a] (±0.28)	6.9 [b] (±0.038)
Site 4 (un-sieved)	21.00 [b] (±2.6)	10.67 [a] (±0.59)	68.33 [b] (±1.07)	2.30 [b] (±0.162)	1.699 [c] (±0.50)	9.9 [c] (±0.069)
Site 5	10.33 [a] (±0.38)	12.00 [a] (±1.20)	77.67 [a] (±1.50)	1.48 [a] (±0.006)	1.44 [a] (±0.04)	7.5 [b] (±0.078)

* Treatments with different letters are significantly different (P<0.05)

4.5 Discussion

4.5.1 Relative roles of river sediment and water for PNP degradation in aquatic systems

The PNP biodegradation rate (Figure 4.5, Table 1.1) revealed that inoculum material did significantly affect the biodegradation potential, and therefore, impacted the effect on the outcome of PNP biodegradation experiments. Figure 4.5 showed that the initial lag phase, in which microbial communities developed, was shorter with sediment present when compared to river water alone, which resulted in reduced degradation times. This is potentially attributable to a higher abundance of microbes in the sediment system due to the presence of the sediment and its additional influx of nutrients.

The study found that the presence of sediment within the experimental units increased the speed of chemical biodegradation, which is in keeping with the findings of other studies (Bradley *et al.,* 2001, Kool, 1984; Kowalczyk *et al.*, 2014). As such, experimental tests used to model biodegradation in the absence of sediment are not truly reflective of the natural riverine environment and merely a reflection of the worst-case scenario of biodegradation due to the increased time taken for biodegradation to occur with water alone. However, as the cut off rates for chemical persistence exceed the time taken for degradation to occur in all experimental units, it can be argued that the test differences irrelevant. Conversely, it could draw attention to the fact that the chemical persistence values currently employed in these standardised tests need evaluating. It is, therefore, potentially worth assessing the environmental accuracy of current chemical persistence levels for different test substances, as more biodegradability tests may need to be conducted with heightened levels of environmental realism, such as seen in OECD test 308 where sediment and water are employed (Thomas and Hand, 2011), to enhance the quantification of test failure rates. This is necessary as degradation of chemicals in the environment affects exposure and, therefore, is a key parameter for assessing

the risk of long-term effects on biota. Degradation rates, or half-lives, can be more accurately determined in simulation tests conducted under representative conditions for the specific environmental compartment (CHEMICALS, 2005).

Moreover, the experimental design identified that the sediment is the driving force behind the biodegradation processes occurring in the experimental units, as the inoculum present in the sediment was clearly responsible for the majority of the biodegradation occurring within the system. This can be seen through the lack of significant difference in biodegradation between the treatments that used river water and sterile water. As the biodegradation rates were comparable between these two treatments, it became apparent that the microbial community present in the sediment predominated the biodegradation processes, meaning that this is the most active layer within the experimental system. These findings supported the initial hypothesis, which assumed that the presence of sediment in the inoculum material does affect the rate of biodegradation. Therefore, the microbial communities that are associated in the highest fluxes of water movement into the sediment/water boundary (hypoeric exchange) are the most important. These are not considered in regulatory chemical degradation OECD tests, although this work suggests these factors should be considered.

4.5.2 Impact of light and dark conditions on the rate of PNP degradation

Previous work, such as that of Kowalczyk *et al.* (2014), identified that light possesses the capacity to impact the biodegradation of PNP in river water due to algal growth. This study has shown that light not only impacts the biodegradation of PNP in river water, but also in river water and sediment systems. Algae inhibited the biodegradation of PNP in river water and river water and sediment systems (Figure 4.6 and Table 4.2). Similarly to Figure 4.5, Figure 4.6 shows that the lag phase in which microbial communities establish

themselves within the system prior to commencement of chemical biodegradation is longer without sediment presence. This suggests that the sediment potentially enables microbial communities to establish faster due to their added nutrient availability and surface area, particularly as the rate of degradation after commencement is comparable (Figure 4.6). Additionally, the effect of light was correlated with changes in river water pH (Figure 4.7) and the proliferation of algae (Figure 4.8) that was detected after light incubation.

The presence of light has the capacity to induce growth of algal biomass in the experimental test systems, thereby inhibiting biodegradation due to the increase in pH associated with photosynthesis (McKendry, 2002). Direct algal suppression of the PNP degrader bacterial communities, and thereby the loss of community biodegradation potential, may also cause the inhibition of PNP biodegradation. Irrespective of the fact that slightly alkaline conditions have been identified as more favourable for effective PNP biodegradation (Li *et al.,* 2008), reductions in PNP biodegradation rate were observed when pH 8.0 was surpassed. This is in keeping with the European Commission datasets that identify the potential of pH to affect the PNP hydrolysis and photodegradation (url:http://esis.jrc.ec.europa.eu). There are studies that have identified that algal presence as a result of light availability increases the biodegradation of crop protection products (Lima *et al*., 2003; Thomas and Hand**,** 2011). However, this study has shown that PNP biodegradation is inhibited by light availability in both water and water and sediment systems alike, which mirrors similar findings in other studies (Kowalczyk *et al.*, 2014).

Light availability has been found to promote algal growth in the test systems with and without sediment. This algal growth caused increases in pH that prohibited the biodegradation of PNP. This finding conflicts with the vast majority of studies that have been conducted to identify the direct effect of light on chemical biodegradation. Similar to this study, other tests have found light incubation to promote algal development. However, unlike this study, they have not found the presence of algae to suppress the biodegradation kinetics. Most

research has shown that light promotes chemical biodegradation due to growth of phototrophic organisms capable of chemical biodegradation (Borde et al., 2003; Davies et al., 2013; Roldán et al., 1998; Li et al., 2005; Lima et al., 2003; Thomas and Hand, 2011). It is for these reasons that light is a concern when conducting biodegradation studies, as interference from increased dissolved oxygen may occur due to the photosynthesis conducted by algae (Xu *et al*., 2005). Moreover, algae can affect photolysis rates of chemicals in water because of light induced algal photosynthesis. This has been shown in studies where chemical compounds underwent accelerated light-dependent photo degradation due to the presence of green and cyanobacteria (Zepp and Schlotzhauer, 1983).

4.5.3 Inter site variability on degradation rate of PNP

This study attempted to identify whether sediment origin and sediment composition impacted the rate of biodegradation in experimental tests, thereby assessing the accuracy of the OECD test protocols. These protocols stipulate the need to employ two completely different sediment types from the same water column to gauge an accurate biodegradation timeline for the test watercourse, following sieving of the sediments to a uniform particle size (< 2 mm), thereby removing the natural diversity of the river bed material. Figure 4.10 is illustrative of the fact that different sediments have different chemical biodegradation potential, which is likely attributable to the variance in the microbial communities which inhabit them. This is supported by other studies that have found that different sediment bacterial composition possessed varying biodegradation potential (Guo *et al.,* 2005). The natural diversity in river sediment types may result in microbial inocula that differ between types of material, which has the capacity to impact biomass and community composition, consequently affecting functional capabilities.

Figure 4.10 also illustrates variance in rate of biodegradation between sites 4 and site 5 when compared to the other sample sites, and site 4 unsieved when compared to all other sampling sites. Additionally, Figure 4.10 demonstrates

variance in lag time before the commencement of biodegradation, with different sites possessing varying start times even though there is no significant difference between the rate of biodegradation. This is reflective of the biodegradation rates measured in Figures 4.5 and 4.6, suggesting that sediment type has the capacity to impact microbial community development and, in turn, biodegradation lag phase.

Figure 4.11 and 4.12 shows that site 4 unsieved is significantly different from the sediments taken from other sample sites, with a significantly higher level of chlorophyll-a and higher biomass seen in the PLFA analysis. However, it is not possible to attribute this difference in composition solely to the lack of application of the OECD test regulatory sieving process that was not implemented on that sample, as site 1 unsieved has no significant variance to any of the other sample site properties. Notwithstanding this, Table 4.3 shows variance in the sediment composition of the sediments from the different sample sites. It indicates lower sand and higher clay content in sediments from site 4, 4 unsieved and 5, all of which degraded PNP significantly slower than the sediments from the other sample sites. This suggests that sediment structure plays a role in biodegradation, potentially due to the sediment composition impacting the microbial distribution and community configuration throughout the sediment. Therefore, the sieving process implemented on sediment samples to standardise OECD tests in the pursuit of uniformity impacts the chemical composition of the sediment. This is because OECD tests lack a definition for inoculum source and quality, which has been identified as the predominant cause of inconsistent test results in water-sediment studies (Vázquez-Rodríguez *et al*., 2008).

The test series showed, through employment of Tukey post hoc tests, that there was a significantly slower rate of PNP biodegradation when the sediment was not sieved at site 4. This means that the OECD test protocols alter the microbial community composition and sediment structure of the samples to a point where the results are not directly reflective of the actual riverbed ecosystem. This is highlighted in Figures 4.11 and 4.12, which demonstrate how significantly different this unsieved sample is from the other sediments.

This is likely attributable to the fact that the surface sediment of the sample was much more densely compacted, with a higher level of microbial community on the surface (and in turn a higher level of chlorophyll-a). These parameters meant that if the sediment was sieved and manipulated to a uniform particle size, the structural integrity and microbial composition of the sediment would have changed, thereby resulting in different rates of biodegradation, as seen in Section 4.4.3. Moreover, sieving the sediment is likely to have resulted in increasing the microbial activity in the biomass in addition to increasing the surface area. This, considered with the lower sand and higher clay content of site 4 unsieved, potentially explains the slower biodegradation rate observed. The test protocols (in terms of sieving) and inoculum origin, therefore, appear to be the driving force behind chemical biodegradation, which is supported by some authors' belief that selection of an appropriate inoculum material may impact a biodegradation test's ability to obtain reliable biodegradation results (Jürgens *et al*., 2009).

Chapter 5

General discussion

To date, there has been a lack of scientific exploration regarding the interactions between bed-form, microbial biofilm communities and chemical biodegradation within the hyporheic zone. This PhD project aimed to enhance understanding of the pollutant dissipation mechanisms that may occur in flowing river systems, in doing so, it provides avenues to increase the environmental realism of regulatory chemical testing. This study's exploration of the hyporheic exchange processes happening in rivers, and its measurement of the impact microbial community development has on this exchange, provides an insight into the lotic system from which assumptions can be made on the way in which microbial biofilm communities influence the environmental mechanics of flowing water systems. Moreover, this study tried to expand the understanding of sediment-water interactions by assessing the impact that sediment type has on chemical biodegradation, in order to evaluate the relevance of regulatory tests. The major findings of the current project are discussed below.

5.1 Reproducibility and Functionality of Experimental System

Hyporheic exchange experimentation has been predominately based in laboratory re-circulating flume studies to permit control of experimental variables (Elliott and Brooks, 1997; Marion *et al.,* 2002; Richardson and Parr, 1988; Tonina and Buffington, 2007). As such, each experimental laboratory system is built to precise specifications to suit its particular interests, capacity, and needs (Williams, 1970; Muste, 2010). Provisional testing is required in all flume tests to ensure reproducibility, functionality and representativeness of the experimental system prior to testing. The primary focus of the flume establishment was to ensure mass balance was achieved in the systems, and that the experimental components had no impact on hyporheic exchange or

biofilm development, creating identical channels which were capable of identification of the impact of specific variables (biofilm development and bed-form) on hyporheic exchange.

The provisional experimentation undertaken found that mass balance could be achieved in the system, with plateaus being seen in the trace tests within 5% of the expected end concentration with the employment of Rhodamine WT as a tracer in an experimental system that employed a synthetic substrate and compensated for temperature fluctuations (Figure 2.24). Although the use of these substrates and experimental controls deviated from complete environmental realism/comparability, their employment in previous studies and utilisation as modeling tools for environmental assessment meant that the data could be extrapolated to provide quantitative expectation of biodegradation potential levels (Chandler, 2013; Lai et al., 1994; Nagaoka and Ohgaki 1990; O'Connor and Harvey, 2008; Richardson and Parr, 1988; Smart and Laidlaw, 1977).

Moreover, the experimental design permitted accurate assessment of the desired experimental variables, removing external factors that may have influenced hyporheic exchange, such as chemical sorption (Figure 2.22) and temperature (Figure 2.21). More importantly, initial flume tests showed the system to be functional in terms of its accuracy in quantifying differences in hyporheic exchange rates, with faster exchange being recorded in large sediments (5 mm sediment particle size) due to the ease of solute transport into the larger interstitial porous spaces when compared to small sediments (0.5 mm sediment particle size), due to decreased porosity and increased particle density inhibiting exchange in the smaller sediment systems (Figure 3.12). Similarly, the accuracy and suitability of the experimental systems to measure hyporheic exchange in river systems was supported by the recorded differences in exchange rates between flat and undulating test series. These provisional tests showed faster rates of exchange in the undulating bed systems, illustrated in Figures 3.13 and 3.14. These findings mirrored the findings reported in major flume hyporheic exchange studies that found identical trends (Bottacin-Busolin

et al., 2009; Elliott and Brookes, 1997a; Marion *et al.,* 2002; O'Connor and Harvey, 2008; Vörösmarty *et al.*, 2010).

5.2 Interactions between bed morphology, sediment particle size, biofilm development and hyporheic exchange in re-circulating flume systems

Chapter 3 highlighted the fact that quantification of hyporheic exchange has been a key focus of research, resulting in copious amounts of analytical assessment, both *in-situ* an *ex-situ* (Battin and Sengschmitt, 1999; Bottacin-Busolin *et al.,* 2009; O'Connor and Harvey, 2008; Marion *et al.,* 2002). However, this work has been fixated on physical parameters that drive exchange within environmental systems, without considering the activity and impact of microbiological phenomena in these systems due to the complexity surrounding environmentally accurate testing.

In its simplest form, *ex-situ* experimentation in a static water system illustrated a significant difference in microbial development with sediment particle size and with increased levels of recorded effective depth penetration of microbial biofilm community in sediments with particle sizes larger than 1.5 mm (Figure 3.15; Table 3.2). This finding supported other work that has shown that environmental variables may have the capacity to impact sediments via clogging of interstitial porous spaces, thereby influencing the rate of exchange (Packman et al., 1997; Nogaro *et al.,* 2010). However, the studies that have previously been conducted to quantify the effect of sediment variability, flow and bed-form on hyporheic exchange have hindered ecological processes that modify the organization and function of the hyporheic zone due to the environmental complexity of the systems. This made it difficult to accurately pinpoint the driving force behind changes in exchange, thereby requiring the *ex-situ* research that this project has undertaken (Hendricks, 1996; Fischer et al., 2005; Nogaro *et al.,* 2010).

Utilising river water as inoculum, biofilm development, quantification and impact on hyporheic exchange was assessed in the re-circulating flume systems. This process enabled the identification of trends in microbial community

development in/on sediment beds. Figures 3.16, 3.17 and 3.18 show that particle size is directly proportional to the extent of biofilm penetration into the bed, with significantly lower amounts of biofilm developing at depth in the 0.5 mm test systems (Figure 3.18). Figure 3.20 illustrates that this microbial biofilm community development had a direct relationship on hyporheic exchange in the small 0.5 mm sediment system, with surface biofilm development occurring as a mat that clogged the interstitial porous spaces causing a reduction in hyporheic exchange over time. Conversely, the figures also show that there was no significant reduction in the 5 mm system, which is likely, attributable to the larger pore spaces not being clogged. This finding extends the work of Nogaro *et al.,* 2010, more accurately quantifying the impact of sediment particle size on hyporheic exchange due to the removal of external variants. Therefore, it can be seen that sediment clogging plays a substantial role in impacting hyporheic exchange when a small sediment particle size is present.

Analysis of biofilm development also identified a variance in the quantities and dispersion of biofilm development on the surface and at depth in undulating systems. Figure 3.25 quantifies the variance in biofilm community development at depth in all test systems. The figure identifies that there was a shift microbial community development when undulations are present in the bed-form. In flat bed systems, there was a uniform biofilm development across the surface of the 0.5 mm sediment and a uniform distribution throughout the entire bed-form of the 5 mm sediment (Figures 3.16 and 3.17). This is in keeping with research expectations as the majority of transport in those systems is driven by molecular diffusion (Chow, 1959; Marion *et al.,* 2002). However, the undulating bed-forms demonstrate variance in microbial development across and throughout the sediment bed-form indicating disparities between biofilm development at different slope sections. The pumping occurring in the system channeled the flow through specific areas of the sediment bed, resulting in increased levels of biofilm development in those areas, predominately the up slope and peak sections where pumping occurs, driving nutrients through the bed (Figure 3.25). The carbohydrate dispersion throughout the bed also indicated that a heightened frequency of bed-forms (i.e. smaller dune wavelength) results in a higher concentration of carbohydrates at depth. However, testing showed that

even with the variance in biofilm community development on undulating beds, it was still the sediment particle size that impacted the rate of hyporheic exchange, as the development of the biofilm either clogged the interstitial pore spaces (0.5 mm sediments) or surrounded the particles but did not have a significant effect on the rate of hyporheic exchange (5 mm particles).

Effectively, the tests demonstrated that microbial development is sediment dependent. If the porosity of the sediment and interstitial pore spaces are small enough to be clogged, biofilm development will dominate the surface and form a mat, which inhibits the rate of hyporheic exchange. However, larger sediments are too dispersed to enable biofilm formation to clog the pore spaces, thereby alleviating any impact the microbial community may possess on exchange. Further testing is therefore required to identify the sediment particle size boundary, at which biofilm development will impact and impede hyporheic exchange.

Moreover, it should be noted that these findings possess huge applications for chemical and pharmaceutical industries. With microbial community development possessing the capacity to inhibit hyporheic exchange, it is likely that chemicals and solutes entering the water column will pass through the environmental compartments faster than they would normally be expected to as they will not be able to penetrate into the sediment bed due to microbial biofilm clogging. This has ramifications on modeling software employed to predict chemical spill extent, lag time and impact as the reduction in hyporheic exchange and infiltration could reduce chemical lag time in the system and extend the reach of the chemical due to its increased speed down the channel. Conversely, the increased exposure of chemicals to microbial communities as they penetrate into the sediment bed, could lead to an increased rate of chemical degradation due to higher contact with the bacterial community.

5.3 Assessment of the transformation rate of p-nitrophenol in aquatic sediment systems; utilizing inter sample comparison to evaluate spatial variability of chemical degradation rates

Regulatory quantification of chemical persistence and subsequent potential hazardous effects to the environment are assessed via employment of standardized biodegradation tests, the majority of which are designed and implemented by the OECD. These tests are designed for use under standardised conditions, minimising environmental variability in test parameters to reduce test expenditure and facilitate experimental reproducibility (Thomas and Hand, 2011). However, the result of these protocols is standardised tests that may not be reflective of the natural environment. Chapter 4 of this study attempted to quantify the suitability of such an approach and assess its appropriateness considering the variance found in sediment bed-form composition along watercourses.

Environmental realism is becoming ever more important in chemical fate assessment, with biodegradation tests being designed in a way that enables the investigation of chemical fate reflective of the specific environmental compartment of interest. OECD test guidelines state that biodegradation studies are used to identify whether the chemical will undergo biodegradation in the environment. The majority of current testing focuses on establishing the successful biodegradation of a chemical, solely quantifying chemical decomposition as opposed to assessing the environmental realism of the laboratory based tests. To improve the accuracy of biodegradation studies, environmental realism needs to be improved, with considerations needing to be taken to account for the impact of natural conditions, such as light/dark cycle, light intensity, bacterial community structure and functions present in inocula, with their variability and consequences regarding the interpretation of biodegradation test results. The Chemical Industry should only use results obtained in more environmentally realistic studies for better prediction of chemical fate in the environment, chemical risk assessment and management.

Chapter 4 undertook experimentation incorporating more realistic conditions than are used in current OECD tests. This involved studying the biodegradation of PNP under light and dark conditions and the impact of inoculum origin on chemical fate. This work identified that algal growth due to light presence inhibited PNP biodegradation in all experimental systems, irrespective of water source or inoculum type. Although application of light in biodegradation test systems could increase the environmental realism, light conditions lead to unpredictable test results. This is because incubation under light conditions promotes algal growth in small-scale closed experimental systems, altering the pH within the experimental unit, thereby influencing the chemical uptake and biodegradation rate of bacterial populations. Such a scenario would not take place in open systems like rivers unless they were heavily eutrophied. Furthermore, experimentation identified that sediment origin and treatment has the capacity to directly impact chemical biodegradation of PNP. This study found that there was little influence of sediment origin or processing method on biodegradation rate of PNP, suggesting that these factors do not need to be accounted for in OECD biodegradation studies (predominately OECD test 308). However, the implementation of sieving within these experimental systems significantly altered the rate of chemical biodegradation. This is because the sieving action alters the sediment particle size distribution and microbial community structure, thereby impacting the readily degradability potential of the sample. The significant increase in biodegradation rates recorded in unsieved sediments with high sand content illustrates the described impact. This is important as inoculum characterization is also beneficial since it provides the comparison of test results obtained with inocula sampled from different environmental compartments. Forney *et al*. (2001) indicated that inoculum density might be used to predict chemical biodegradation, since high inoculum density is related with higher abundance of specific degraders. Therefore the alteration of the inoculum sample, through the process of sieving to standardise the test format, will impact the test results. Hence, it would be useful to further investigate the fate of this chemical compound in river water, sediment and soil and, therefore, determine more realistic experimental parameters for PNP biodegradation, thus assessing the impact of sediment sieving and sand content on biodegradation.

5.4 Impact of Sediment Composition on Experimental Research

This project has identified the impact that sediment type possesses on hyporheic exchange (Chapter 3), identifying sediment particle size as an influential factor in terms of its impact on biofilm development. The tests showed that microbial community development differs in depth and distribution on sediment beds with a small sediment particle size. Further experimentation, assessing the accuracy of OECD test standardisation, showed that modification to sediment treatment and structure has a direct impact on biodegradation (Chapter 4). These tests have subsequently identified the overwhelming importance that sediment size and treatment plays within the riverine system (Ngora *et al.,* 2011), with particle size and structure impacting microbial development on sediment beds, whilst sediment treatment methodology influences biodegradation. This experimental identification of influence seen from the different types of tests undertaken in this project highlights the need for further investigation into sediment impact in degradation studies, assessing the sediment structure, shape and microbial composition to enhance the environmental realism of degradation tests. Figure 5.1 shows these findings identifies potential future experimental needs to further our understanding of sediment-water interactions in lotic systems.

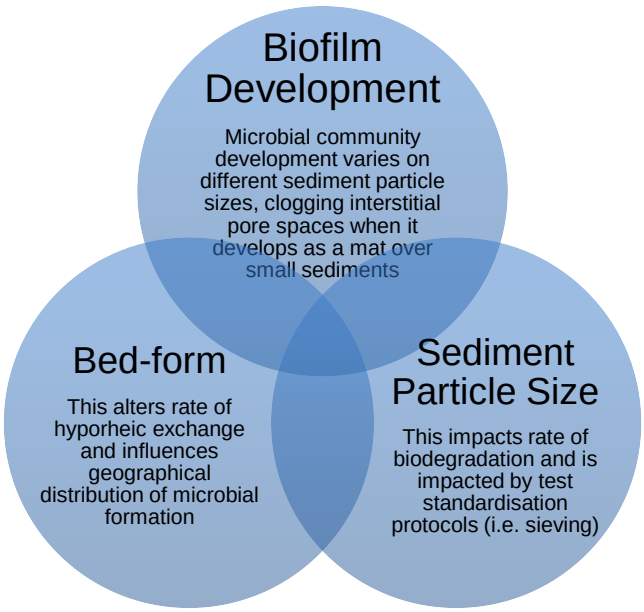


Figure 5.1: Schematic showing integration of findings from current research

Microbial adaptation and biodegradation processes play a key role in chemical fate in the environment. Persistence of chemicals in the natural environment is generally assessed through biodegradation studies with microbial inocula. The awareness of sediment influence on microbial community development and biodegradation highlights the importance of inoculum source and treatment on the quantification of biodegradation. However, limited work has been conducted on these factors due to the complexity associated with adding environmental realism to test systems, the variability of sediments seen in the natural environment and associated costs of undertaking more detailed tests.

5.5 Future Recommendations

Presently, datasets from biodegradation studies could be applied to models to stimulate different environmental scenarios that could improve our understanding of processes occurring in the environment and aid in prediction of chemical persistence. Figure 5.1 highlights the overlap between this project's findings, identifying a potential research area for test expansion. Increased knowledge on behaviour of chemicals in response to the environmental variables investigated (predominately sediment size and bed-form) would be beneficial for chemical risk assessment conducted by the Chemical and Pharmaceutical Industry.

An example of such methodological employment is the mechanistic model currently being developed at Unilever SEAC to simulate the OECD 314E test system. The model couples water quality parameters (COD, NH_3) and microbial growth (heterotrophic and autotrophic biomass) with chemical fate to establish more accurate degradation kinetic parameters to quantify in-river exposure modeling. Further experimental work is required to enable the model to better characterize the environmental parameters found in most systems. Similarly to this, continuation of the work conducted in this project has the capacity to enhance our understanding of microbial community development in rivers, with great potential to generate enhanced chemical fate modeling through the

furthered knowledge of the effect of sediment and bed-form associations on chemical fate, thereby permitting chemical companies, such as Unilever, to gain a more robust understanding of their potential impact on the environment.

Provisionally, the endeavor to enhance understanding of chemical fate in river systems can be achieved by furthering our understanding of the sediment dynamics. This project has shown that microbial development differs greatly between sediment particle size and topographically over varying bed-formations. To enable a more robust understanding, additional work needs to be undertaken on biofilm clogging of interstitial pore spaces. The lack of knowledge of microbial community development on different sediments limits our ability to predict its rate of development in the natural environment. This study quantified the rate of biofilm development on two different uniform particle sizes, however further quantification needs to be undertaken to identify the variance seen between different sediments, both in terms of particle sizes and heterogeneity, thereby providing a more varied understanding of sediments impact on microbial development and therefore exchange.

5.6 Future Work

The interdisciplinary nature of this thesis has provided insight into the potential research opportunities that exist to enhance and develop the current understanding of microbial development implications on hyporheic exchange, whilst simultaneously generating opportunity to further bridge the knowledge gap between fluid dynamics and microbiology by identifying the degradation kinetic implications of microbial development on river beds. Thereby creating opportunity to further the environmental realism seen in degradation studies.

The tests conducted in this investigation permitted advancement towards inclusion of additional levels of environmental realism in degradation tests, whilst breaking new ground in the scientific understanding of microbial development in river systems. There are numerous topics that could be

considered for further investigation based on the research presented in this thesis. A number are outlined below:

- The utilisation of real substrates extracted from river beds in the test system, as opposed to the use of synthetic substrates, would add another level of environmental realism to the experimental systems. The heterogeneity seen in actual river sediments, in terms of sediment particle size, sediment structure, microbial community diversity and organic content, is likely to undergo a much more complex series of responses when hyporheic exchange and chemical degradation is assessed as a result of the variance in microbial community development.
- Following on from the incorporation of real sediments into the flume systems, testing methodology conducted in Chapter 3, to quantify microbial development and its impact on hyporheic exchange, could be modified to combine the degradation experiment with the flume exchange tests. This would quantify the temporal chemical degradation in a flowing water system, thereby identifying if water movement has the capacity to impact chemical degradation. This would permit comparison with standardised regulatory testing to identify the accuracy of static OECD tests. However, it should be noted that the large quantities of water and chemicals required to conduct a flume test would require careful consideration for chemical waste disposal.
- Assessment of light and/or dark incubation on chemical biodegradation of PNP in the re-circulating flume systems. This would enable identification of the impact of flowing water conditions on chemical degradation under different light conditions to quantify its affects and identify whether PNP degradation is inhibited to the same extent as that seen in this study (Chapter 4).

- Time restraints within the study (due to equipment issues and experimental establishment) prevented mixed diameter sediment beds from being investigated. These are more representative of the sediment beds found in natural streams, enabling investigation of the effects of

surface armouring on hyporheic exchange and the variation in the diffusion coefficient within the bed.

- Chemical sorption to sediment particles was not included in this investigation due to the employment of a synthetic substrate and a chemical tracer with low sorptive properties. Irrespective of this fact, chemical sorption is an important factor within pollutant modeling that should be considered. Using natural sediments in the experimental system to quantify the biodegradation of specific chemicals would permit the effects of chemical sorption to be quantified within the re-circulating flume systems. The use of chemicals would require developing novel measurement techniques to allow concentrations to be measured within the bed sediment, which could prove to be particularly difficult at depths.

The supplementary investigations suggested above have the potential to add several levels of environmental realism to the experimentation that can generate conditions more closely comparable to those within natural steams and permit monitoring of the exchange and biodegradation of chemical pollutants within the laboratory. These modifications, if proven successful, could then be used to enhance current regulatory tests, such as refining OECD 308 [OECD, 2002] tests for biodegradation, which does not incorporate sediment origin or fluid flow. These modifications have the capacity to lead to a more realistic evaluation of chemical persistence in the natural environment through the generation of a more realistic test paradigm.

5.7 Project Summary

The environment has been, and may continue to be, detrimentally affected due to the intertwined nature of ecosystems with anthropogenic activity (Carlow and Petts, 1992; Vitousek *et al.*, 1997). Recently, there has been an increase in the awareness regarding household chemical utilization and the subsequent influx of these substances into the environment due to the potential adverse human and ecological effects resulting from their employment and disposal (Lapertot and Pulgarin, 2006).

To predict environmental fate of chemicals, the Organisation for Economic Cooperation and Development (OECD) have established standardised laboratory methods to estimate environmental biodegradation in river systems. The simulation tests are designed to provide data for chemical biodegradation rates under specified environmentally relevant conditions. The established tests incorporate indigenous biomass, media, or relevant solids (e.g. soil, sediment) to replicate a particular set of environmental conditions. These tests are designed to employ biodegradation kinetics that mimics the actual environmental conditions more closely. However, there are numerous variables which may impact the results of these tests due to environmental variation, such as adaptation and concentration of microorganisms found in riverine environments; as such the established tests could be considered inaccurate. Additionally, biofilms, hyporheic exchange and other physical properties (transport, diffusion and pumping) have been hypothesized to have a significant impact on the biodegradation of such chemicals.

To date, there are few studies that address the interactions between bed-form, microbial biofilm communities and chemical biodegradation at the sediment-water interface. To reach a stage where enhanced modelling can be conducted, experimentation and data collection is required. This investigation aimed to explore the effect of bed-form characteristics on the diversity and pollutant degrading potential response of microbial biofilm communities at the sediment-water surface, and the subsequent impact biofilm development and bed-form

has on hyporheic exchanges. The main objectives are highlighted in Chapter 1, Section 1.9.

Using flume systems, the effects of bed-form and sediment particle size on hyporheic exchange were investigated in an attempt to add environmental realism to experimental testing. Initially, work was conducted to identify the reproducibility of the flume systems, ensuring that the exchange recorded was in keeping with findings of similar tests and literature (Chapter 2). These tests showed that the flume system used in the study was capable of recording differences in exchange rates between different bed-forms and varying particle sizes (Figure 5.2, work described in Chapter 3). The demonstration of faster rates of hyporheic exchange in sediment systems with larger sediment particle sizes and faster exchange in systems with bed-forms present are in-keeping with other flume studies due to the nature of hyporheic exchange (Elliott and Brookes, 1997a; O'Connor and Harvey, 2008).

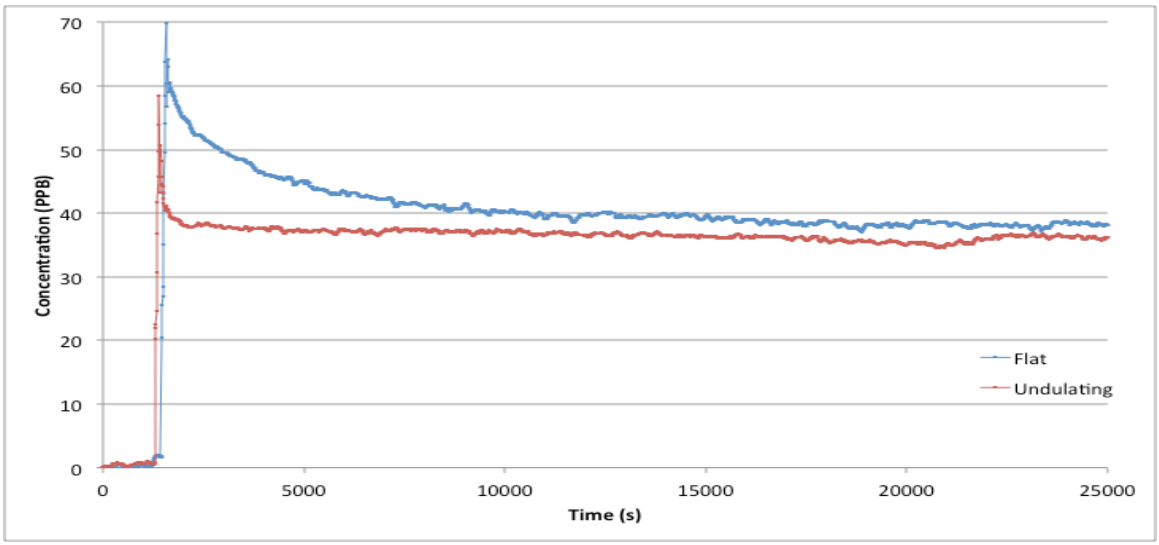


Figure 5.2a: A visualisation demonstrating the variation of the rate of hyporheic exchange due to varied bed form

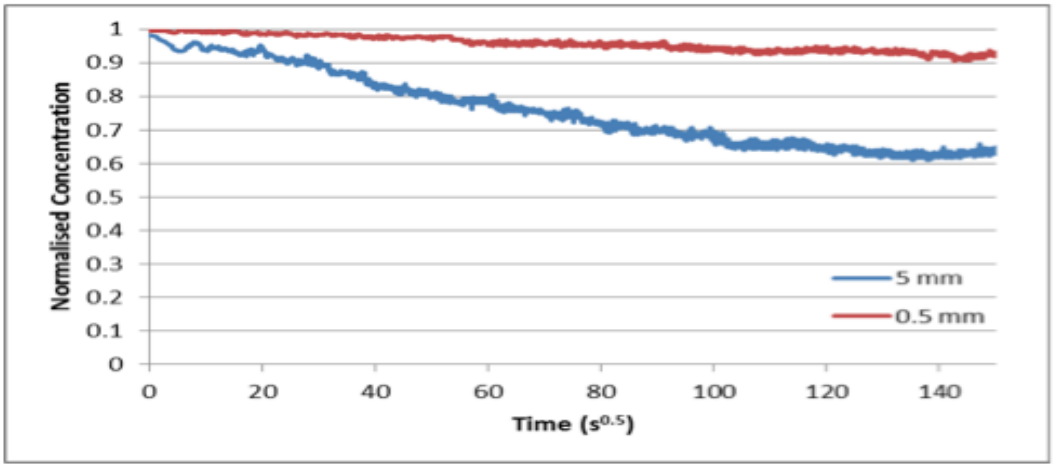


Figure 5.2b: Demonstration of normalised rate of exchange in the experimental systems following initial dye injection at Time 0, demonstrating the variation of hyporheic exchange using different bead sizes

The flume tests showed that variation of sediment size has an impact on the rate of hyporheic exchange in the system due to differences in the development of microbial communities on the differing sediments (Chapter 3, Section 3.4.3). This study identified that microbial development only has the capacity to reduce the rate of hyporheic exchange in 0.5 mm sediments due to clogging of the interstitial porous spaces in the sediment at the surface layer, illustrated in Figure 5.3. As test duration increased, subsequently resulting in growing abundance of surface biofilms on the 0.5 mm sediment bed, the rate of hyporheic exchange reduced, irrespective of bed-form (Figure 5.4).

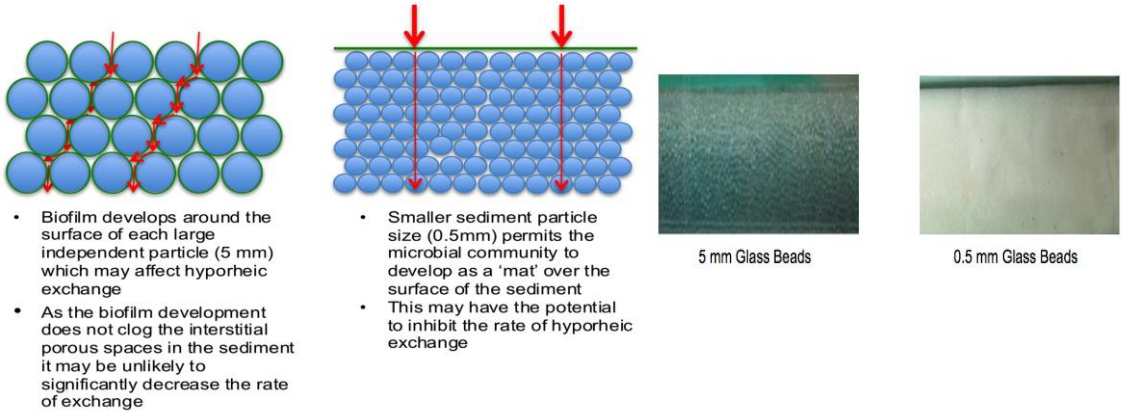


Figure 5.3a: Illustration of differences between potential biofilm developments on different sediment particle sizes

5 mm glass beads

0.5 mm glass

Figure 5.3b: Recorded differences in biofilm developments on different sediment particle sizes during flume tests. Figure illustrates concentrated biofilm development on surface of 0.5 mm sediment

This variance in biofilm development was quantified via analysis of carbohydrate presence, both in terms of quantity on the surface and that at depth, which is indicative of biofilm presence due to the secretion of carbohydrates by the biofilm EPS (Chapter 3, Section 3.4.4). The analysis showed that there was significantly more carbohydrate at the surface layer of the 0.5 mm sediment, with all the biofilm concentrating there over the duration of the test. This was significantly different to the spread of carbohydrate distribution in the 5 mm sediment bed, which distributed in a more uniform manner throughout. This difference in biofilm distribution resulted in a significant temporal reduction in hyporheic exchange rate in the 0.5 mm sediment tests which had high surface levels of biofilm, irrespective of bed-form (Figure 5.4). Figure 5.4 also shows that the biofilm development in the 5 mm sediment did not significantly impact the rate of hyporheic exchange, suggesting that the smaller the sediment particle size, the greater the chance of sediment clogging by biofilms.

Utilising river water as inoculum in the hyporheic exchange tests provided an insight to the variance seen in microbial community development in sediment beds, with variance between the effective depth penetration of the biofilm in the sediment bed dependent upon the particle size and sediment bed-form. Additionally, this study enabled quantification of the impact of microbial community development on hyporheic exchange, identifying that the clogging of interstitial porous spaces of fine sediments by biofilms inhibits the rate of exchange, furthering the work of Nogaro *et al.,* 2010. The accuracy,

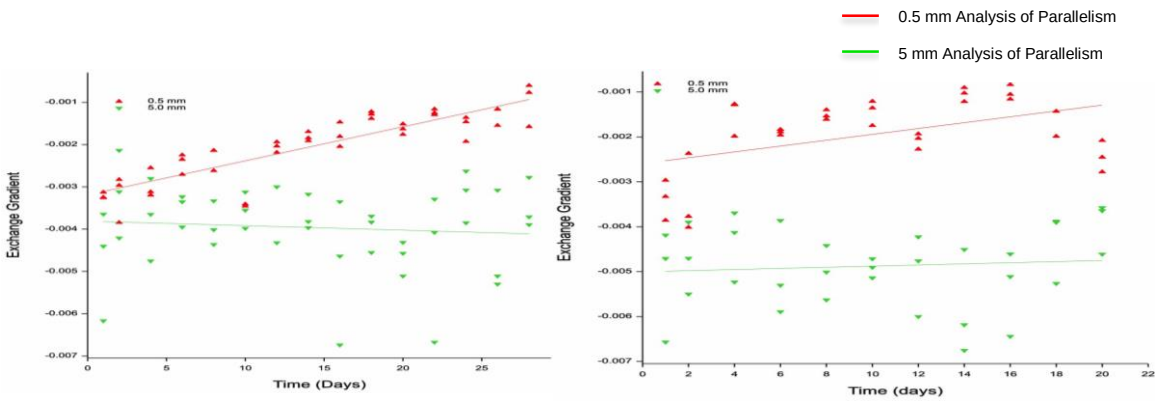


Figure 5.4: Temporal variance in normalised hyporheic exchange gradients from flatbed flume tests (left) and undulating bed flume tests (Right); taken at 30 % exchange

reproducibility and repeatability of the experimental flume systems were supported by data obtained in this current study (Chapter 3).

To enhance the accuracy of environmental studies that quantify the rate of chemical biodegradation in the fluvial environment, implementing chemical degradation tests in flowing water flume systems under realistic environmental conditions would be beneficial. In this project, work was conducted in an attempt to set a foundation for this experimental progression by undertaking established OECD tests but implementing environmental variables to identify if these possess an impact on the rate of biodegradation, assessing the effects of light, the origin and treatment of the sediment utilised (sieving). This was done via measurement of PNP degradation and the production of Chlorophyll-a, isolating and quantifying the effect of light, sediment treatment and sediment origin on the biodegradation of PNP. This investigation is detailed in Chapter 4.

Initially the effect of sediment presence and absence was tested on the biodegradation of PNP and subsequently, light was introduced as a variable (Figure 5.5). The results demonstrate the key role that sediment plays in the degradation of PNP, thus reflecting the role of sediment and associated microbial community in the bioremediation of household chemicals that enter the sewage and subsequent water systems (Section 4.4.1). This study has also shown that the presence of light during incubation not only impacts the

biodegradation of PNP in river water, but also in river water and sediment systems. Furthermore, the presence of light has encouraged the growth of algae (Figure 5.6). This algal growth caused increases in pH that prohibited the biodegradation of PNP.

Finally, the impact of sediment origin and sieving of sediment before testing (as stipulated in OECD guidelines for tests) was investigated and shown to have a significant effect on community structure (Chapter 4, Section 4.4.3). This is evident from looking at the variance in Chlorophyll concentrations and rate of biodegradation due to treatment and site location (Figure 5.7).

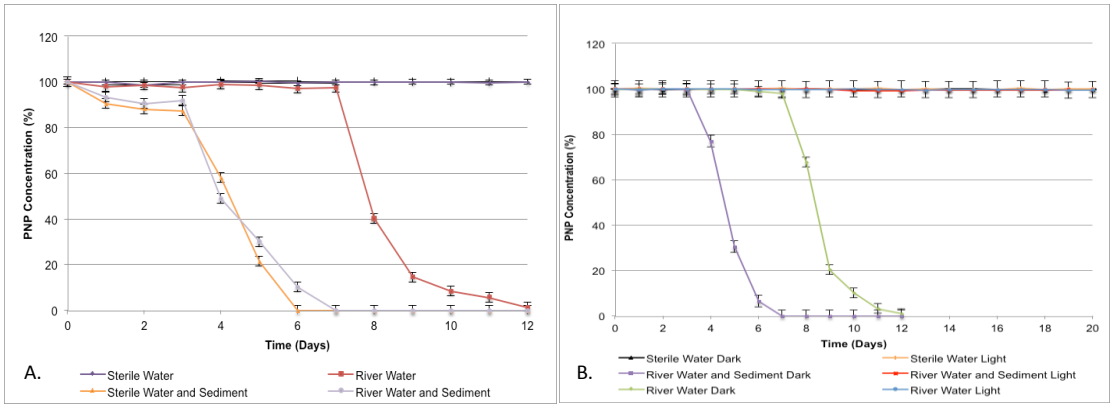


Figure 5.5: Illustration of PNP degradation under varying conditions. A. River water treatments incubated in dark conditions with & without sediment. B. River water treatments incubated in light and dark conditions with & without sediment

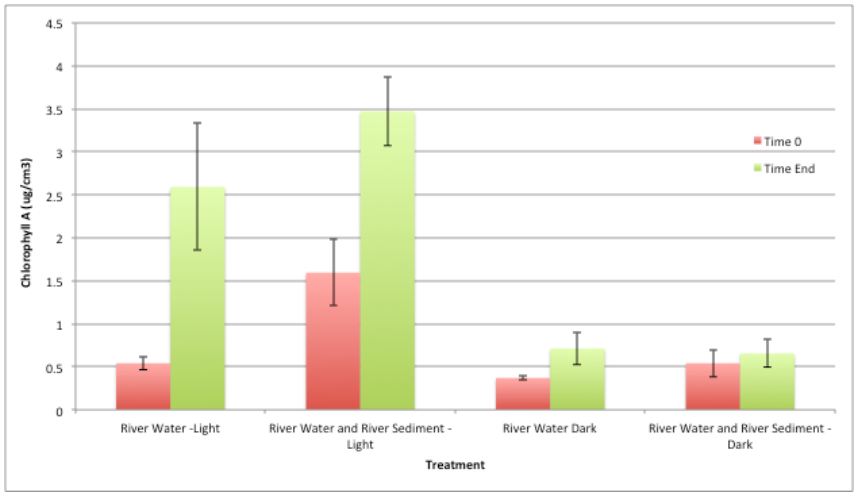


Figure 5.6: Mean chlorophyll-a variation in experimental units during testing under employment of different light conditions. Error bars represent ± standard error of the mean

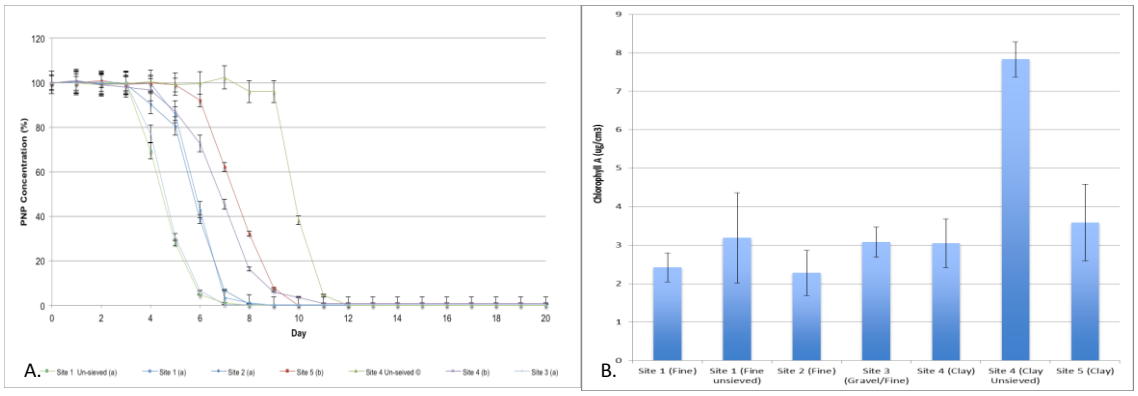


Figure 5.7: A. The biodegradation of PNP under employment of different sediment types taken from the River Dene. Error bars represent ± standard error of the mean. * Treatments with different letters are significantly different (P<0.05) B. Chlorophyll-a content of sediment from different sample sites along the River Dene. Error bars represent ± standard error of the mean.

The OECD test protocols stipulate the need to employ two completely different sediment types from the same water column to gauge an accurate biodegradation timeline for the test watercourse, following sieving of the sediments to a uniform particle size (< 2 mm), thereby removing the natural diversity of the river bed material (Chapter 4, Section 4.5.3).

This project is illustrative of the fact that different sediments have different chemical biodegradation potential, which is likely attributable to the variance in the microbial communities that inhabit them. Moreover, the project has demonstrated that sediment particle impacts the rate and spatial location at which microbial communities develop. As such, it is of pivotal importance to add environmental realism to current tests so that a more accurate understanding of degradation kinetics in river systems is better understood. Essentially, the proposition of advancement of the flume testing undertaken in this project to incorporate chemical degradation studies utilizing real substrates, in addition to monitoring impacts of light, temperature and flow, has the capacity to dramatically increase the accuracy of chemical persistence evaluation.

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Appendices

a.1 Nitrate Test

Nitrate levels were measured daily to identify change in nutrient levels; used to quantify biofilm development via depletion of nutrients (Costerton *et al.*, 1995).

Method:

- HACH low range nitrate test was used
- One viewing tube was filled with sample to be tested, stoppered and shaken. The tube was then emptied and the process repeated
- The colour viewing tube was then filled with sample
- Nitrate reagent powder was added to the sample, stoppered and shaken for three minutes
- The sample left to settle for 30 seconds to permit unoxidised particles of cadmium metal to settle to the bottom of the sample
- The prepared sample was poured into the second colour viewing tube to separate from the cadmium particles.
- A nitrite reagent pillow was added to the sample, stoppered and shaken. A red colour would have developed if nitrate was present
- After 10 minutes the sample was inserted into the top right opening of the colour comparator.
- The first sample tube was emptied, rinsed and then filled with water and placed into the second opening of the colour comparator
- The comparator was held up to a light source and a colour match obtained, generating the mg/l nitrate for the sample

a.2 Phosphate Test

Phosphate levels were measured daily to identify changes in nutrient levels. Water samples were taken every 48-hours and analyzed using HACH testing.

Method:

- Both viewing tubes were filled with 5 mL of the sample water
- Four drops of Ammonium molybdate reagent were added to one of the tubes, stoppered and mixed
- Phosphate 2 powders were added to the same tube, stoppered and inverted repeatedly until dissolved
- If phosphate is present the sample turned turn blue
- The tube of prepared sample and the tube of untreated water were placed in the colour comparator
- The comparator was held up to a light source and a colour match obtained, generating the mg/l nitrate for the sample

a.3 Nutrient Monitoring

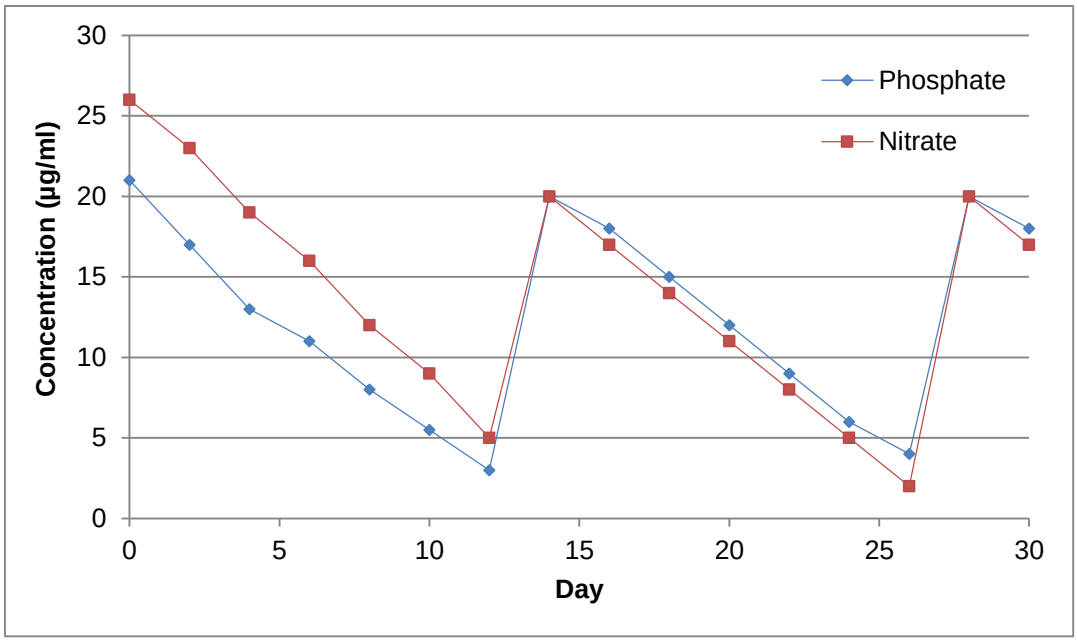

Figure A.1: Temporal recorded variance in nitrate and phosphate levels in flat bed glass bead test, and the system's response to addition of sodium nitrate and potassium orthophosphate

Equations Utilised in analysis

Molecular diffusion coefficient is calculated by taking porosity and tortuosity into account, thereby generalising the sediment molecular diffusion expression to:

$$D'_m = \beta D_m \quad \text{1}$$

Where β signifies the empirical expression for tortuosity as a function of porosity (θ) as described by Iversen and Jørgensen (1993) (O'Connor and Harvey, 2008; Equation 2).

$$\beta = \frac{1}{1+3(1+\theta)} \quad \text{2 → Equation 3 from O'Connor and Harvey (2008)}$$

Sediment Equations Used:

Porosity (dimensionless) is calculated by:

$$\theta = \frac{Volume\ of\ Voids}{Total\ Volume}$$

$$= \frac{Water\ weight\ in\ Voids}{Total\ water\ weight}$$

$$= \frac{Wet\ weight\ of\ sediment\ in\ container - Dry\ weight\ of\ sediment}{Total\ water\ weight} \quad \text{3}$$

$$K = 5.6 \times 10^{-3} \frac{\theta^3}{(1-\theta)^2} \quad \text{4 → Equation 30 from O'Connor and Harvey (2008)}$$

$$ks = 3d_{90} + 1.1\Delta(1 - EXP^{-\frac{25\Delta}{\lambda}}) \quad \text{5 → Equation 31 from O'Connor and Harvey (2008)}$$

Velocity Equations:

$$U = \frac{Q}{Channel\ Width * Flow\ Depth} \quad \text{6}$$

$$u* = \sqrt{G\ R\ S_o} \quad \text{7}$$

$$\text{Estimated } u* = 1/10\ U \quad \text{8}$$

$$R = \frac{Surface\ Area}{Wetted\ Perimeter} \quad \text{9}$$

$$S_o = \frac{Change\ in\ elevation\ of\ flume}{Flume\ Length} \quad \text{10}$$

Equations used for comparative analysis with O'Connor and Harvey (2008)

$$D_e = \left(\frac{\sqrt{\pi}}{2}\frac{V_w}{A_s}\frac{dC^*}{d(t^{1/2})}\right)^2 \quad \text{11 → Equation 11 from O'Connor and Harvey (2008)}$$

$$A_s = Channel\ Length \times Channel\ Width \quad \text{12}$$

$$Re^* = u_* \frac{k_s}{\upsilon} \quad \text{13}$$

$$Pe_k = u_* \frac{\sqrt{K}}{D'_m} \quad \text{14}$$

$$X - axis\ value = Re_* Pe_k^{6/5} \quad \text{15}$$

$$Y - axis\ value = \frac{D_e}{D'_m} \quad \text{16}$$